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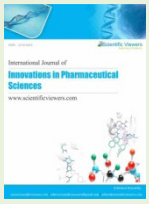

A STUDY ON ANTI-DIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF DIOSPYROS MALABARICA KOSTEL BARK ON ALLOXAN INDUCED DIABETIC IN RATS

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ABSTRACT

The aim of the present study was to investigate antidiabetic activity of ethanolic extract of *Diospyros malabarica* bark with its antioxidant potential. Effect of ethanolic extract of *Diospyros malabarica* bark at a dose level of 100mg/kg and 200mg/kg on blood glucose, serum triglycerides, serum total cholesterol, HDL cholesterol and also for the levels of antioxidant enzyme such as superoxide dismutase (SOD), catalase and lipid peroxidation (LPO) in pancreatic homogenate was studied on alloxan induced diabetic rats. Ethanolic extract of *Diospyros malabarica* bark at a dose level at dose of 100 mg/kg and 200 mg/kg treated groups showed considerable improvement in all the parameters studied in a dose dependant manner. Antihyperglycaemic action of ethanolic extract of *Diospyros malabarica* bark may be due to antioxidant potential of extract which is revealed by improvement in the levels of antioxidant enzymes in pancreas of alloxan diabetic rats and validate its claim in Indian system of medicine.
KEYWORDS: *Diospyros malabarica*, antidiabetic, antioxidant enzyme, alloxan.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder and is major public health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death and third when all its fatal complications are taken into accounts. It is characterized by high blood glucose concentration (hyperglycemia) caused by insulin deficiency often combined with insulin resistance[1]. Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis. When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which in turn results in dehydration, thirst and increased drinking of water (polydipsia).

The most characteristic feature in DM is persistent high level of glucose in the blood. When glucose cannot be metabolized by the cells then it remains in the blood stream and a person with diabetes therefore has constantly high blood glucose levels. As the glucose level becomes sufficiently high some of the glucose is excreted in the urine. Hence, the name 'diabetes' means 'to run through' and 'mellitus' means sweet or with a taste of honey[2].

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost.

The plant *Diospyros malabarica* belongs to the family Ebenaceae. Traditionally, the bark of this plant was reported as astringent, acrid, cooling, anti-inflammatory, constipating, depurative and febrifuge, and is useful in vitiated conditions of Pitta, burning sensation, diarrhoea, dysentery, leprosy, skin diseases, pruritus, dyspepsia, haemorrhages, burns, diabetes, fever, spermatorrhoea and vaginal disorders[3]. As per the literature survey information, aqueous fruits extract of Diospyros lotus (family: - Ebenaceae) showed antidiabetic effect[4]. In view of the above information the present study has been undertaken to evaluate the anti-diabetic activity methanolic extract of bark of the plant Diospyros malabarica.

MATERIALS & METHODS

Plant material

The plant *Diospyros malabarica* was collected from Tirupati, Andhra Pradesh, India identified and authenticated by Dr. K. Madhava Chetty assistant professor in Department of Botany Sri Venkateswara University Tirupathi.

Preparation of Extract

The bark of the plant was washed and cleaned. Then the bark was shade dried at room temperature. Dried bark were powdered and packed in air tight container. The coarse material was subjected to successive soxhlet extraction by using ethanol solvent. The extract was concentrated under reduced pressure and stored in desiccators for complete removal of solvent. The percentage yield was calculated. Hence forth this ethanolic, extract of *Diospyros malabarica* will be called as EDM [5].

Phytochemical Analysis

The conventional chemical tests were carried out for the extract of Ethanolic *Diospyros Malabarica* to identify the presence of various chemical constituents like alkaloid,tannin,c-glycoside, reducing sugar and tri terpenes.

Animals

Adult Wistar albino rats (150-180 g) of either sex were procured from the animal house, from Albino Research and Training Institute, Hyderabad, Andhra Pradesh, India and used in the study. The animals were kept under standard environmental conditions of room temperature (220 ±20C), relative humidity (50% ± 5%) and 12 h light and dark cycle. The animals were housed in the colony cages (either three rats or six mice per cage) and provided feed (commercial pellets contain a balanced ration obtained from the Rayans Biotechnologies Pvt. Ltd., Hyderabad) and water ad libitum.

All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water. All the experiments were carried out in accordance with the guidelines of Industrial Animal Ethics Committee (IAEC). The study was conducted after obtaining ethical committee clearance from the IAEC.

Acute oral toxicity studies

Animals showed good tolerance to single doses of *Diospyros malabarica* ethanol extract) in doses as high as 2 g/kg and were non-lethal. We selected 200 mg/kg of ethanolic extract of *Diospyros malabarica* to test the antidiabetic effect. Further the dose of ethanolic diospyros malabarica did not produce any noticeable signs of toxicity (behavioral changes) and mortality after once daily administration for fifteen-days.

Effect of Ethanolic extract of Diospyros malabarica bark on alloxan diabetic rats[6]

Rats were divided into 10 groups consisting of 6 rats in each group. The rats were acclimatized for a period of 7 days before starting the experiment. After overnight fasting, hyperglycaemia was induced by administering a single dose of alloxan monohydrate supplied by s.d Fine-Chemical Ltd. Mumbai, India (150 mg/kg) prepared in sterile saline to all the groups except group I which served as normal control. During this period, the animals were given free access to water. After 5 days of alloxan administration, fasting blood glucose levels of rats were checked by glucostrips. The animals having blood glucose levels > 250 mg/dl were separated and selected for further studies and then re-grouping of these hyperglycemic rats was done as per the following protocol, five groups each group containing six animals for studying the antidiabetic activity of the extract

Group I- Normal Control (sterile saline)
Group II- Diabetic Control (Alloxan monohydrate)
Group III- Glibenclamide (5 mg/kg,)
Group IV- Low dose of ethanolic extract of *Diospyros malabarica* (100 mg/kg)
Group V- High dose of ethanolic extract of *Diospyros malabarica* (200mg/kg)

The treatment was started from the same day except normal control and diabetic control groups for a period of 15 days orally. During this period, animals in all groups had free access to standard diet and water. Blood glucose levels were estimated on 1st, 4th, 7th and 15th day of the treatment where blood samples were collected through retroorbital plexus puncture. On the 16th day the animals were anaethesized by mild ether anaesthesia. Blood was collected and allowed to stand for one hour, serum was separated by centrifuging and evaluated for different biochemical parameters. Like

A. Serum Glucose Levels [7]
B. Lipid Profile
    i. Serum Total Cholesterol Levels [8]
    ii. Serum Triglycerides Levels

iii. Serum HDL Cholesterol Level

C. Endogenous enzymatic and non-enzymatic antioxidant levels
   i. Lipid peroxidation[9]
   ii. Catalase[10]
   iii. SOD[11]

Statistical analysis

The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's 't' test.

RESULTS

Effect of Ethanolic extract of Diospyros malabarica bark on alloxan diabetic rats:

The antihyperglycemic potential of Ethanolic extract of *Diospyros malabarica* was observed. The extract (100 mg/kg & 200 mg/kg p.o.) exhibited significant dose dependent decrease in blood sugar ($p<0.001$) after seven days of treatment in rats, when compared to alloxan elevated blood glucose level. The standard drug glibenclamide (10 mg/kg p.o) exhibited significant decrease in blood sugar ($p<0.001$) at seven days of treatment. Results are shown in Table 1 and Graph representing the effect of EDM on Alloxan induced diabetic in rats in Graph 1.

Effect of Ethanolic extract of Diospyros malabarica bark on triglycerides:

The triglycerides level in serum significantly increased ($p<0.001$) in alloxan treated animals when compared to control.Total cholesterol levels of animals treated with Ethanolic extract of *Diospyros malabarica* (100 mg/kg & 200 mg/kg p.o) and glibenclamide (5 mg/kg p.o.) showed significant decrease ($p<0.001$) when compared to alloxan treated animals. Results shown in Table 2 and Graph representing the effect of EDM on Alloxan induced diabetic in rats in Graph 2.

Effect of Ethanolic extract of Diospyros malabarica bark on total cholesterol:

The total cholesterol level in serum significantly increased ($p<0.001$) in alloxan treated animals when compared to control. Total cholesterol levels of animals treated with Ethanolic extract of *Diospyros malabarica* (100 mg/kg & 200 mg/kg p.o) and glibenclamide (5 mg/kg p.o.) showed significant decrease ($p<0.001$) when compared to alloxan treated animals. Results shown in Table 3 and Graph representing the effect of EDM on Alloxan induced diabetic in rats in Graph 3.

Effect of Ethanolic extract of Diospyros malabarica bark on HDL Cholesterol:

The HDL cholesterol level in serum significantly decreased ($p<0.001$) in alloxan treated animals when compared to control. Total HDL cholesterol levels of animals treated with Ethanolic extract of *Diospyros malabarica* 100mg p.o. ($p<0.05$), 200 mg/kg p.o ($p<0.001$) showed significant increase when compared to alloxan treated animals. Glibenclamide (5 mg/kg) showed significant increase ($p<0.001$) when compared to alloxan treated animals. Results are shown in Table 4 and Graph representing the effect of EDM on Alloxan induced diabetic in rats in Graph 4.

Effect of Ethanolic extract of Diospyros malabarica bark on Superoxide Dismutase (SOD):

The superoxide dismutase (SOD) level in pancreas significantly decreased ($p<0.001$) in alloxan treated animals when compared to control. SOD levels of animals treated with Ethanolic extract of *Diospyros malabarica* 100mg/kg p.o & 200 mg/kg p.o and glibenclamide (5 mg/kg p.o.) showed significant increase ($p<0.001$) when compared to alloxan treated animals. Results are shown in Table 5 and Graph representing the effect of EDM on Alloxan induced diabetic in rats in Graph 5.

Effect of Ethanolic extract of Diospyros malabarica bark on Catalase (CAT):

The catalse (CAT) level in pancreas significantly decreased ($p<0.001$) in alloxan treated animals when compared to control. Catalase levels of animals treated with Ethanolic extract of *Diospyros malabarica* 100 mg/kg p.o & 200 mg/kg p.o and glibenclamide (5 mg/kg p.o.) showed significant increase ($p<0.001$) when compared to alloxan treated animals. Results are shown in Table 6 and Graph representing the effect of EDM on Alloxan induced diabetic in rats in Graph 6.

Effect of Ethanolic extract of Diospyros malabarica bark on Lipid peroxidation:

The lipid peroxidation level in pancreas significantly increased ($p<0.001$) in alloxan treated animals when compared to control. Lipid peroxidation levels of animals treated with Ethanolic extract of *Diospyros malabarica* 100 mg/kg p.o & 200 mg/kg p.o and glibenclamide (5 mg/kg p.o.) showed significant decrease ($p<0.001$) when compared to alloxan treated animals. Results are shown in Table 7 and Graph representing the effect of EDM on Alloxan induced diabetic in rats in Graph 7.

Table 1: Effect of administration of Ethanolic extract of *Diospyros malabarica* bark on blood glucose level of alloxanized diabetic rats on 15th day.

Treatment and Doses	Blood glucose concentration(mg/dl)			
	1st day	4th day	7th day	15th day
Control (Normal Saline)	90.5 ± 3.3	90.7±4.6	90.8±2.5	90.8±2.5
Diabetic control (Alloxan) 150mg/kg	265.6±16.4	263.5±17.5	270.6±10.6	275.4±12.3
Glibenclamide 5mg/kg	261±13.3	230±10.5	130±9.5	94.5±8.5
Ethanolic extract 100mg/kg	263±15.0	220.2±13.5	180.4±12.6	168.7±10.5
Ethanolic extract 200mg/kg	267±18.2	170.5±12.9	140.6±11.6	105±8.9

Values are mean ± SEM of 6 rats in each group,*p<0.001 day 15 are compared with 1day

Table No 2: Effect of Ethanolic extract of *Diospyros malabarica* bark on Triglyceride

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide [5mg/Kg]	Group IV EDM [100mg/Kg]	Group V EDM [200mg/Kg]
Triglyceride (mg/dl)	78.04± 0.88	116.58± 0.17a*	93.70± 0.23 b*	113.29± 0.11b*	99.08± 0.23b*

Values are mean ± SEM of 6 animals in each group, Comparison between: a - Group I and Group II, b – Group II and Group III, IV, V. Statistical difference are expressed as * p<0.001

Table No 3: Effect of Ethanolic extract of *Diospyros malabarica* bark on Total cholesterol

Group	**Group I** Control	Group **II** Diabetic Control	Group **III** Glibenclamide **[5mg/kg]**	**Group IV EDM [100mg/kg]**	Group **V EDM [200mg/kg**
Total Cholesterol in Serum (mg/dl)	84.03± 0.36	241.6± 0.42a*	149.06± 1.37b*	135.8± 0.71b*	105.50± 0.16b*

Values are mean ± SEM of 6 animals in each group, Comparison between: a - Group I and Group II, b – Group II and Group III, IV, V. Statistical difference are expressed as * p<0.001

Table No 4: Effect of Ethanolic extract of *Diospyros malabarica* bark on HDL

Group	**Group I** Control	Group **II** Diabetic Control	Group **III** Glibenclamide **[5mg/kg]**	**Group IV EDM [100mg/kg]**	Group **V EDM [200mg/kg]**
HDL Cholesterol in serum (mg/dl)	52.97± 0.46	30.2± 0.45a**	54.03± 0.20b**	44.8± 0.24b*	48.01± 0.12b**

Values are mean ± SEM of 6 animals in each group, Comparison between: a - Group I and Group II, b – Group II and Group III, IV, V. Statistical difference are expressed as * p<0.05; ** p<0.001

Table No 5: Effect of Ethanolic extract of *Diospyros malabarica* bark on Superoxide Dismutase

Group	**Group I** Control	Group **II** Diabetic Control	Group **III** Glibenclamide **[5mg/kg]**	**Group IV EDM [100mg/kg]**	Group **V EDM [200mg/kg]**
Superoxide Dismutase in pancreas (Units/mg protein)	28.61± 0.068	10.60± 0.019a*	23.71± 0.033b*	16.81± 0.029b*	21.68± 0.020b*

Values are mean ± SEM of 6 animals in each group, Comparison between: a - Group I and Group II, b – Group II and Group III, IV, V. Statistical difference are expressed as * p<0.001

Table No 6: Effect of Ethanolic extract of *Diospyros malabarica* bark on Catalase (CAT)

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide [5mg/kg]	Group IV EDM [100mg/kg]]	GROUP V EDM [200mg/kg]
Catalase (CAT) in pancreas (nmole of H_2O_2 decomposed/min/ mg of protein)	32.61± 0.030	12.29± 0.021[a*]	26.96± 0.031[b*]	18.53± 0.026[b*]	25.15± 0.061[b*]

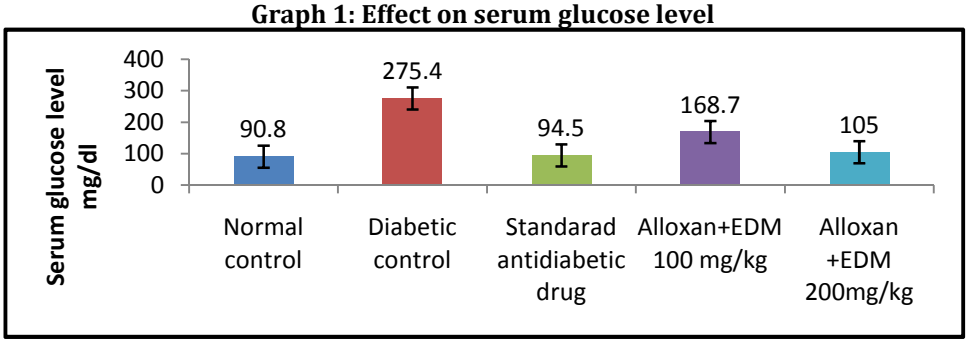
Values are mean ± SEM of 6 animals in each group, Comparison between: a - Group I and Group II, b – Group II and Group III, IV, V. Statistical difference are expressed as * p<0.001

Table No 7: Effect of Ethanolic extract of *Diospyros malabarica* bark on Lipid peroxidation

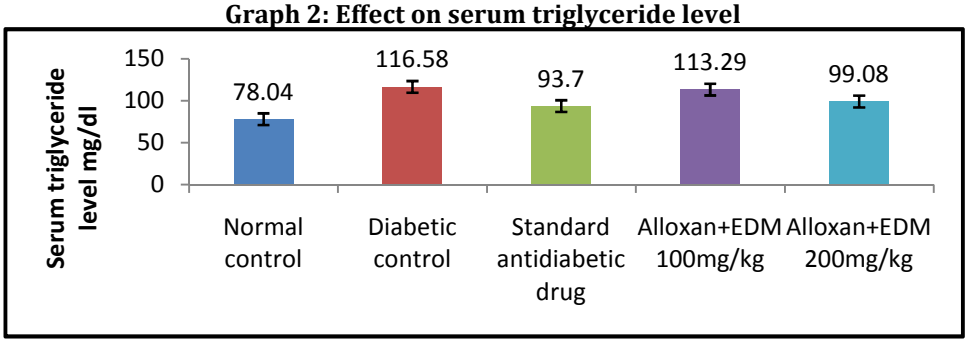
Group	**Group I** Control	Group **II** Diabetic Control	Group **III** Glibenclamide **[5mg/kg]**	**Group IV EDM [100mg/kg]**	Group **V EDM [200mg/kg]**
Lipid peroxidation in pancreas (n mole of MDA formed/ min/mg of protein)	5.69± 0.025	17.30± 0.024[a*]	6.10± 0.027[b*]	8.11± 0.01[b*]	6.83± 0.028[b*]

Values are mean ± SEM of 6 animals in each group, Comparison between: a - Group I and Group II, b – Group II and Group III, IV, V. Statistical difference are expressed as * p<0.001

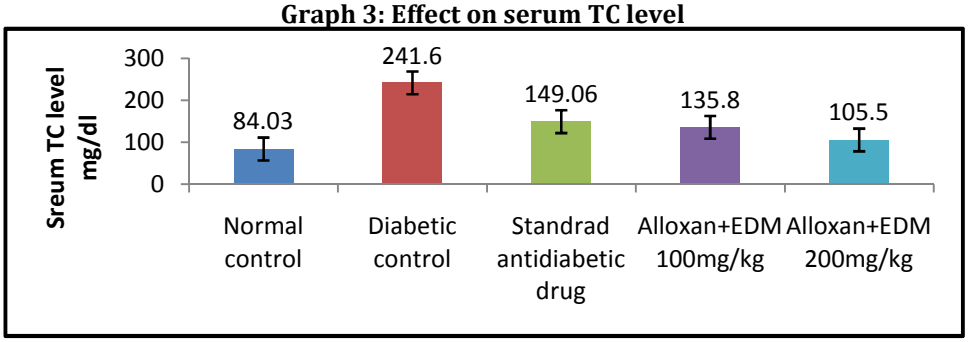
Graph 1: Effect on serum glucose level



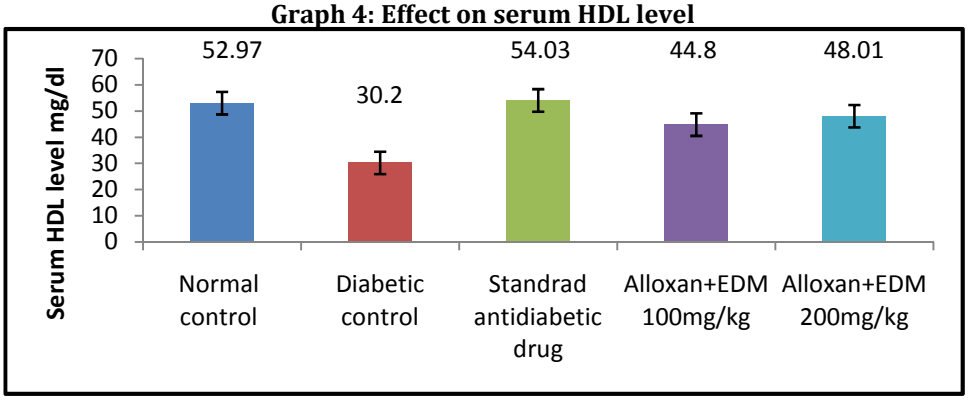
Graph 2: Effect on serum triglyceride level



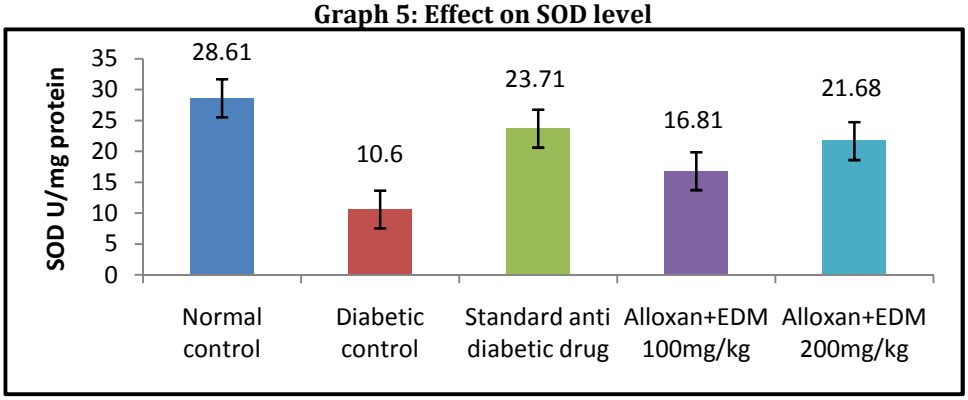
Graph 3: Effect on serum TC level



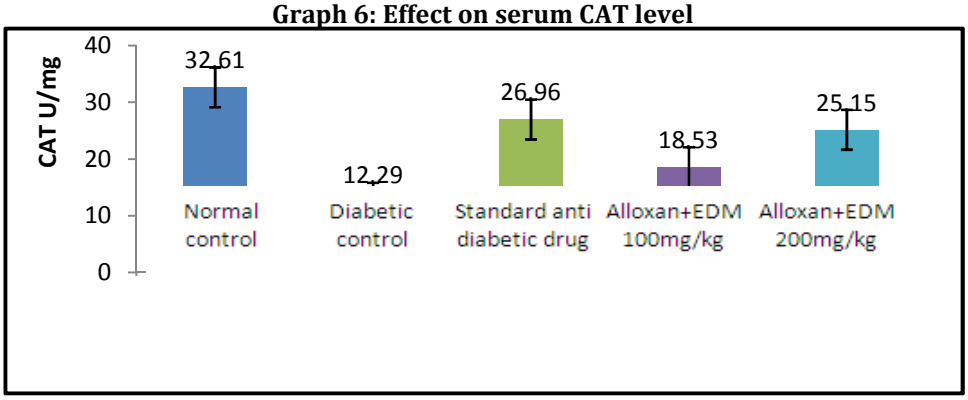
Graph 4: Effect on serum HDL level



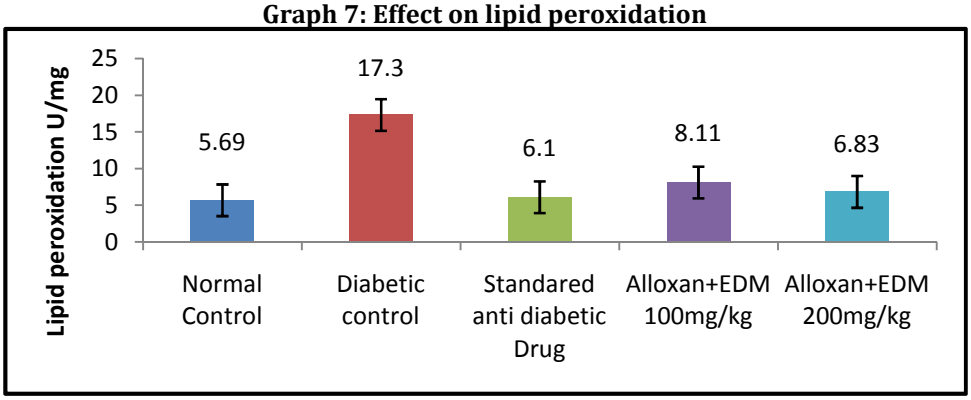
Graph 5: Effect on SOD level



Graph 6: Effect on serum CAT level



Graph 7: Effect on lipid peroxidation



DISCUSSION

Management of diabetes without any side effects is still a challenge to the modern medicine. This leads to increasing the demand for searching new drugs from natural origin with antidiabetic and free from side effect or less side effects. In Ayurvedic or indigenous folk medicines, the hypoglycemic plants have been in use generally in their natural forms (fresh juice, paste or dry powder). These include both the inorganic and organic constituents of the concerned herbs. Further it is important to note that the inorganic part of a medicinal plant containing mainly mineral elements sometimes plays a contributory role in enhancing medicinal properties (including hypoglycemic activity) of that plant.

Effect of Ethanolic extract of Diospyros malabarica bark on blood glucose level of alloxanized diabetic rats:

Alloxan is widely used to induce diabetes in experimental animals. In alloxan diabetes rats the blood glucose levels were in the range of 260-275mg/dl,which were considered as severe diabetes. In the standard drug Glibenclamide(5mg/kg) and ethanolic extract(100 mg/kg) and (200mg/kg) treated groups,(Table No.1) the peak values of blood sugar significantly decreased to 94.5mg/dl, 172.2mg/dl,105.2mg/dl on the 15th day respectively. Thus,the ethanolic extract (200mg/dl) was found to be almost significant as standard drug lowering blood glucose level, where as the ethanolic extract (100mg/dl) treated group showed blood glucose level that is comparatively less to ethanolic extract (200mg/dl) and standard.

Effect of Ethanolic extract of Diospyros malabarica bark on some biochemical parameters of hyperglycemic rats:

The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG) and HDL in control and experimental animals were investigated (Table-2). Alloxan induced rats showed significantly increased serum lipid profiles except HDL when compared with normal rats. The glibenclamide and ethanol extracts of *Diospyros malabarica* bark treated rats showed a significant decrease in the content of lipid profiles when compared with diabetic induced rats. Similarly HDL level decreased in alloxan induced diabetic rats when compared to normal rats. On administration of ethanol extracts of *Diospyros malabarica* bark and glibenclamide to the diabetic rats, HDL level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents risk factor for coronary heart disease. The hypolipidemic effect may be due to inhibition of fatty acid synthesis. In normal metabolism insulinactivates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after *Diospyros malabarica* bark treatment may be directly attributed to improvements in insulin levels.

Effect of Ethanolic extract of Diospyros malabarica bark on Catalase activity ,Lipid peroxidative and SOD state of panecreas of hyperglycemic rats:

Oxidative stress induced by alloxan has been shown to damage pancreatic β-cells and produce hyperglycemia in rats. Oxidative stress produced by alloxan was found significantly lowered by administration of ethanolic extract *Diospyros malabarica* of, this was evident from significant decrease in the lipid peroxidation in pancreas. Superoxide dismutase, Catalase, glutathione peroxidase constitutes a mutually supportive team of defense against reactive oxygen species.

Superoxide dismutase is a metalloprotein and is a first enzyme involved in the antioxidant defense by lowering the steady state of oxygen radical. In hyperglycemia, glucose undergoes autoxidation and produce superoxides and it produce free radical then in turn leads to lipid peroxidation in lipoprotein. Catalase is a hemeprotein,localized in the peroxisomes or the microperoxisomes, which catalyses the decomposition of hydrogen peroxide to water and oxygen and thus protect the cell from oxidative damage produced by hydrogen peroxide.

CONCLUSION

Ethanolic extract of *Diospyros malabarica* exhibit potent antioxidant, antidiabetic activity in alloxan induced rats. phytochemicals are known to have antidiabetic activity . Thus it can be assumed that tannins or triterpenes present in EDM is responsible for the antidiabetic activity. Revealed a dose dependent antidiabetic potential in rats with doses of 100 and 200 g/kg. The dose of 200 mg/kg b.w /day was found to be having maximum activity, and the effect was seen equal to the levels of blood glucose with standard antidiabetic drug, glibenclamide. Exhibited significantly hypolipidemic effect in rats. However further

chemical and pharmacological investigations are needed to find out the active phytochemical and the exact mechanism of action.

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