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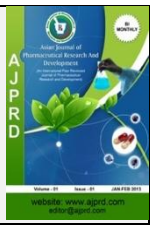

Open Access Research Article

Evaluation of Antidiabetic Potential of *Eucalyptus Globulus* Plant Extract in Dexamethasone-Induced Diabetic Rats

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A B S T R A C T

***Objective*:** Evaluation of Antidiabetic Potential of *Eucalyptus globulus* Plant Extract in Dexamethasone-Induced Diabetic Rats.

***Method*:** Methanolic leaves extract of *Eucalyptus globulus* Plant was prepared by Soxhlet extraction method. Female Albino Wistar rats were made diabetic at the dose of Dexamethasone (5mg/kg/day i.p.) for 12 days. Methanolic leaves extract of *Eucalyptus globulus* Plant (200mg/kg, 400mg/kg & 600mg/kg/day p.o.) was screened for antidiabetic activity. Standard drug Metformin (40mg/kg/day p.o.) was administered to the second group of animals for 12 days. Blood glucose levels and body weights of rats were recorded on 0, 6, 12th days. As well as Hypoglycemic & OGTT evaluation was done. At the end of respective treatment different biochemical estimations & histopathological examination of liver was also carried out.

Result: 12 Days oral administration of the *Eucalyptus globulus* Plant leaves extract caused significant (P< 0.05) reduction in blood glucose level & body weight was also gained as compared with toxic control group. Further, it showed the hypoglycemic activity & significant oral glucose tolerance as compared with control animals. The extract also improved other altered biochemical parameters associated with diabetes. Concurrent histopathological examination of liver of these animals showed regeneration by Methanolic leaves extract which was earlier necrosed by Dexamethasone.

Conclusion: Results obtained in this study substantiate the Antidiabetic potential of Methanolic leaves extract of *Eucalyptus globulus* Plant the source of Ellagitannins a bioactive polyphenol and could be considered for further evaluation in clinical studies and drug development.

Keywords: Antidiabetic potential, OGTT, *Eucalyptus globulus*, Polyphenols, Dexamethasone, Insulin.

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1. INTRODUCTION

Diabetes mellitus is the chronic metabolic & pancreatic islet disorder mainly characterized by disruption in carbohydrates, protein, and fat metabolism caused by an inability to produce insulin or a defect in utilization. The hyperglycemia caused due to decreased insulin production is called Type-1 diabetes and hyperglycemia due insufficient insulin utilization is called Type-2 diabetes. The feature of diabetes mellitus is polyuria, polydipsia, weight gain and polyphagia. It is also characterized by chronic hyperglycemia and glucosuria, caused by an absolute or relative deficiency of insulin. This may result into the development of further complications which include hypertension, atherosclerosis, ketosis, gangrene and microcirculatory disorders. It is also associated with long-term complications including retinopathy, nephropathy, neuropathy and angiopathy[1]. The IDF (International Diabetes federation) has subsequently released estimates of the numbers of people with diabetes for 2003 and forecasts for 2025 of 194 million and 334 million, respectively. India leads the world

with largest number of diabetic subjects earning of term "diabetes capital of the world"[2]. Hyperglycemia can be handed initially with oral synthetic 2 Advances in Pharmacological Sciences agent and insulin therapy. Glucose lowering drugs usually succeed in lowering blood sugar levels, therapeutic agents like Insulin, Sulfonylureas, Meglitinides, Biguanides, Thiozolidinediones, DPP-4 inhibitors, α-Glucosidase Inhibitors, Incretin agonists, D2 Agonist may reduce the risk of type 2 diabetes but healthy lifestyle choices remain essential[3]. However, on chronic usage most of these agents produced several side effects including hypoglycemic coma, insulin resistance, hyper-sensitivity, jaundice, abdominal pain, anorexia and metallic taste. Because of the high mortality and morbidity arising from its attendant complications and problems associated with the use of conventional antidiabetic agents[4]. In the natural system of medicine, many Plants have been claimed to be useful for the treatment of diabetes mellitus. The dependence of large rural population on medicinal Plants for treatment of diabetes is because of its availability and affordability. The current worldwide trends towards utilization of Plant-derived natural remedies have, therefore, created a dire need for accurate and up-to-date information on the properties, uses, efficacy, safety, quality & less cost of medicinal Plant products than the semi-synthetics or synthetics. The Plant kingdom has become a target for the search by multinational drug and biologically active lead compounds. In this regard herbal, ayurvedic remedies can improve diabetic conditions without side effects[5]. Ellagitannins (ETs) and ellagic acid (EA) are polyphenols present in some fruits, nuts and seeds, such as pomegranates, black raspberries, raspberries, strawberries, walnuts, almonds & also present in '*Eucalyptus globulus* Plant'. Ellagitannins contain various numbers of hexahydroxydiphenoyl units, as well as galloyl units and/or sanguisorboyl units bounded to sugar moiety. In order to determine the quantity of every individual unit, the hydrolysis of the extracts with trifluoroacetic acid in methanol/water system is performed. They form a diverse group of bioactive polyphenols with anti-inflammatory, anticancer, antioxidant and antimicrobial (antibacterial, antifungal and antiviral) activity[6]. So, the present study was undertaken to Evaluate Antidiabetic Potential of *Eucalyptus globulus* Plant Extract the source of Ellagitannins in Dexamethasone - induced Diabetic Rats.

2. MATERIALS & METHODS

2.1 Collection of Plant Material:

Eucalyptus globulus Plant was collected from Local area of Kolhapur District, Maharashtra, India in January 2021 and authenticated as *Eucalyptus globulus (Family: Myrtaceae)* by Department of Botany, Yashwantrao Chavan College of Science, Satara, Maharashtra, India based on the taxonomical features of the whole Plant material including Leaves.

2.2 Preparation of Methanolic Extract:

Leaves of *Eucalyptus globulus* Plant were shade dried for one week after proper cleaning. *Eucalyptus globulus* Plant leaves were coarsely grounded & Methanolic leaves extract was prepared using Soxhlet apparatus by hot percolation method. The obtained extract was concentrated to dryness using rotatory evaporator under reduced pressure & low pressure (<40°C). Extract was kept in air-tight container and stored at 4°C for further studies.

2.3 Phytochemical Screening:

The extract was subjected to phytochemical analysis to test the presence of volatile oils, carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, propanoids, sterols terpenoids, ketones & alcohols in the leaves extract.

2.4 Drugs and Chemicals:

Dexamethasone obtained from Merck/Dolphin Pharmacy Instruments Pvt. Ltd. Mumbai. Metformin obtained from Aventis Pharma, Ltd. Goa. ACCU-CHECK Active Glucometer procured from Roche Diabetes Care, India/Dolphin Pharmacy Instruments Pvt. Ltd. Mumbai.

2.5 Animals & Housing condition:

Female Albino Wistar Rats of (150-200gm) were selected for experimental study. The animals were maintained under standard laboratory conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were kept and maintained under laboratory conditions of temperature 22 ± 2°C, relative humidity 50 ± 15% and 12 hrs. light/dark cycle. They were allowed free access to food (standard pellets) and water ad libitum. Experimental protocols and procedures used in this study was approved by the Institutional Animal Ethics Committee (IAEC) of YSPM's, YTC, Faculty of Pharmacy, Satara, Maharashtra, India.

2.6 Induction of Diabetes:

Dexamethasone is a synthetic glucocorticoid prevents postoperative nausea and vomiting but may increase blood glucose. These drugs will promote gluconeogenesis or increased blood sugar levels in blood. Chronic exposure to high doses of Dexamethasone causes insulin resistance.

All the Female Albino Wistar animals except control group were administered with Dexamethasone at a dose of 5mg/kg i.p. once a day for 12 days before 1hr. of test drug treatment.

2.7 Collection of Blood samples, Blood Glucose & Body Weight Determination:

Blood samples were withdrawn from tail tip of rats. Fasting blood glucose estimation and body weight measurement were done on 0, 6 & 12th day of the study. Blood glucose estimation can be done by one touch ACCU-CHECK Active Glucometer using glucose test strips.

On day 12th, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated. After that body weight of animals was determined.

2.8 Biochemical Estimation:

Blood samples were with drawn for estimation of Blood glucose level, Serum Insulin level, Lipid profile (Total cholesterol, Triglycerides, HDL, LDL, VLDL) in the serum sample. After the end of respective treatment, animals were sacrificed with high dose of anaesthesia and the tests organs

were removed, weighed and stored at -20℃ for further antioxidant and histopathological studies.

2.9 Experimental Design:

2.9.1 Acute Toxicity Study:

Acute toxicity study was carried out for the *Eucalyptus globulus* Plant by adapting fixed dose method of CPCSEA, OECD guidelines no. 423. Healthy Female Albino Wistar rats were randomly divided into 4 groups with 3 animals in each group. The animals were kept fasted overnight providing only water, after which the Methanolic leaves extract of *Eucalyptus globulus* Plant were administered orally with increasing doses (100, 500, 1000 and 2000mg/kg/day) by intra gastric tube to determine the safe doses by up and down staircase method. The animals were observed continuously for 1 hr., then frequently for 4 hrs. and later at the end of 24 hrs. for general neurological & behavioural or autonomic profile. Further, one group was administered high dose of *Eucalyptus globulus* Plant leaves extract orally once a day for 15 days and observed for any lethality and death.

2.9.2 Hypoglycemic Evaluation:

For Hypoglycemic evaluation, Female Albino Wistar Rats were used and divided into four groups of six animals in each group. Animals were kept fasted overnight (14hrs.) before treatment.

Group I- (Control) rats received vehicle that was 5% Tween 80 (10ml/kg/day p.o.).

Group II- (Test1) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (200mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Group III- (Test2) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (400mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Group IV- (Test3) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (600mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Blood glucose was estimated on 0, 1, 2, 3 & 4th day of the treatment using the ACCU-CHECK Active Glucometer.

2.9.3 Oral Glucose Tolerance Test:

For OGTT evaluation, Female Albino Wistar Rats were used and divided into five groups of six animals in each group. Animals were kept fasted overnight (14hrs.) before treatment.

Group I- (Control) rats received vehicle that was D-Glucose (2gm/kg p.o.).

Group II- (Standard) rats received Glibenclamide (0.5mg/kg i.p.).

Group III- (Test1) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (200mg/kg p.o.) solubilized in 5% Tween 80 solution.

Group IV- (Test2) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (400mg/kg p.o.) solubilized in 5% Tween 80 solution.

Group V- (Test3) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (600mg/kg p.o.) solubilized in 5% Tween 80 solution.

D-glucose (2gm/kg p.o.) was administered to all the rats after one hour of administration of different treatments. Blood glucose was estimated at 30, 60, 90 & 120 min after D-Glucose treatment using the ACCU-CHECK Active Glucometer.

2.9.4 Dexamethasone-Induced Rodent Model of Diabetes:

The Female Albino Wistar rats were divided into six groups of six rats in each. All the animals were fasted overnight (14hrs.) before the treatment of Dexamethasone. All the Female Albino Wistar animals except control group were administered with Dexamethasone at a dose of 5mg/kg i.p. once a day for 12 days. Standard & test drug treatment was started after 1hr. of Dexamethasone administration till the end of study.

Group I- (Control) rats received vehicle that was 5% Tween 80 solution (10ml/kg/day p.o.).

Group II- (Toxic Control) rats received Dexamethasone (5mg/kg/day i.p.).

Group III- (Standard) rats received Metformin (40mg/kg/day p.o.) solubilized in distilled water.

Group IV- (Test1) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (200mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Group V- (Test2) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (400mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Group VI- (Test3) rats received Methanolic leaves extract of *Eucalyptus globules* Plant (600mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

2.10 Statistical Analysis:

All values of results were presented as mean ± standard error of mean (SEM). The statistical analysis involving two groups was evaluated by means of Student's *t*-test, whereas one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest was used for statistical comparison between control and various treated groups. Statistical significance was accepted at the $p < 0.05$ values.

3. RESULTS

3.1 Quantitative Phytochemical Test:

The yield of extract was found to be 4.8%. The phytochemical analysis revealed that the Methanolic leaves extract of *Eucalyptus globulus* Plant contains a significant amount of volatile oils, carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, propanoids, sterols and terpenoids, ketones, alcohols.

3.2 Acute Toxicity Study:

In the LD50 value determination, we observed that the *Eucalyptus globulus* Plant extract was safe to use in animals. There was no change in neurological, behavioural

or autonomic, no lethality or toxic reactions were found with the selected doses (100, 500, 1000 and 2000mg/kg/day p.o.) until the end of study period. Therefore 200, 400 & 600mg/kg was selected for all in-vivo experiments as maximal dose.

3.3 Hypoglycemic Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant in Normal Rats:

The results from the study clearly indicated that the administration of Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 200, 400 and 600mg/kg/day p.o. reduced the blood glucose level significantly on 4th day as compared with normal control group.

Table 1: Hypoglycemic Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant in Normal Rats

Sr. no.	Groups (n=6)	Fasting Blood Glucose Level (mg/dl)				
		0th day	1st day	2nd day	3rd day	4th day
I	Control	86.00±0.73	85.16±0.65	83.50±1.05	82.83±1.07	82.33±0.49
II	Test group with Low dose of E. g. Plant leaves extract	86.66±1.05	85.16±0.60	81.00±0.57	79.33±0.33	78.50±0.42*
III	Test group with Intermediate dose of E. g. Plant leaves extract	85.83±1.01	84.83±0.79	81.65±0.91	79.83±0.70	78.62±0.42*
IV	Test group with High dose of E. g. Plant leaves extract	86.16±0.70	83.50±0.67	80.16 ±0.47	78.55±0.67	75.33±0.95*

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.4 Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on the Oral Glucose Tolerance Test in Normal Rats:

The results from the study clearly indicated that the administration of Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg p.o.) and Metformin (40mg/kg p.o.) reduced the blood glucose level (hyperglycemia due to glucose load 2g/kg p.o.) significantly after 60 min of administration, as compared with control group.

Table 2: Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on the Oral Glucose Tolerance Test in Normal Rats.

Sr. no.	Groups (n=6)	Fasting Blood Glucose Level (mg/dl) in min				
		0 min	30 min	60 min	90 min	120 min
I	Control	94.17±0.65	98.00±0.74	105.51±1.05	114.84±1.07	121.34±0.49
II	Standard	93.51±0.67	96.17±0.70	89.17±0.47*	88.51±0.67*	85.34±0.95**
III	Test group with Low dose ofE. g. Plant leaves extract	94.17±0.60	96.67±1.05	90.00±0.58	89.34±0.33	88.51±0.42*
IV	Test group with Intermediate dose of E. g. Plant leaves extract	94.84±0.79	95.87±1.01	90.67±0.91	89.84±0.70	88.63±0.42*
V	Test group with High dose of E. g. Plant leaves extract	93.66±1.05	95.74±1.01	89.84±0.70	88.63±0.42	85.94±0.95*

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.5 Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on Body Weight of Diabetic Rats:

At the end of 12 days treatment, body weight was significantly decreased in toxic control group as compared with normal control group & significantly increased in Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) and standard drug Metformin (40mg/kg/day p.o.) treated group as compared with toxic control group.

Table 3: Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on Body Weight of Diabetic Rats.

Sr. no.	Groups (n=6)	Body Weight of Animals (gm)		
		0 day	6th day	12th day
I	Control	176.67±0.77	187.33±0.92	193.00±0.92
II	Toxic control	179.17±0.71	184.50±0.57	169.67 ±0.67
III	Standard	179.00±0.78	182.50±1.06**	184.33±0.81**
IV	Test group with Low dose of E. g. Plant leaves extract	174.33±5.05	187.83±0.95**	189.50±0.35**
V	Test group with Intermediate dose of E. g. Plant leaves extract	179.67±0.50	186.33±1.71**	188.33±0.56**
VI	Test group with High dose of E. g. Plant leaves extract	179.67±0.50	183.33±1.71**	186.67±0.77**

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.6 Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on fasting blood glucose level in Diabetic Rats:

A marked rise in fasting blood glucose level was observed in toxic control group as compared with normal control group. The Methanolic leaves extract of *Eucalyptus globulus* Plant and standard drug Metformin (40mg/kg/day p.o.) treated group which produced a significant reduction in blood glucose level as compared with toxic control group. Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) exhibited a dose dependent significant antidiabetic potential on 6 & 12th days post treatment.

Table 4: Effect ofMethanolic Leaves Extract of *Eucalyptus globulus* Plant on fasting blood glucose level in Diabetic Rats

Sr. no.	Groups (n=6)	Fasting Blood Glucose Level (mg/dl)		
		0th day	6th day	12th day
I	Control	88.50±2.06	88.33±2.09	90.33±1.88
II	Toxic control	265.00±1.17	297.67±1.21	349.67±4.57
III	Standard	265.67±1.32	221.67±2.83**	115.25±1.52**
IV	Test group with Low dose of E. g. Plant leaves extract	265.83±0.78	256.83±0.78**	146.83±0.78**
V	Test group with Intermediate dose of E. g. Plant leaves extract	266.33±2.64	244.67±2.01**	140.67±2.01**
VI	Test group with High dose of E. g. Plant leaves extract	268.33±2.64	235.67±1.02**	129.67±2.01**

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.7 Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on Biochemical Parameters in Diabetic Rats

3.7.1 Serum Insulin Level

After 12 days of treatment period it was observed that decreased serum insulin level in toxic control group as compared with normal control group. Animals treated with Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) and standard drug Metformin (40mg/kg/day p.o.) treated group showed a significant increase in the serum insulin level as compared with toxic control group.

Table 5: Serum Insulin Level

Sr. no.	Groups (n=6)	Serum Insulin Level *(µU/ml)*
I	Control	18.15±0.55
II	Toxic control	7.25±0.31
III	Standard	17.65±0.33**
IV	Test group with Low dose of E. g. Plant leaves extract	12.32±0.37**
V	Test group with Intermediate dose of E. g. Plant leaves extract	13.32±0.34**
VI	Test group with High dose of E. g. Plant leaves extract	16.32±0.39**

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.7.2 Serum Lipid Profile:

After 12 days of treatment period it was observed that increased level of CHL, LDL, VLDL, TG & decreased HDL level in toxic control group as compared with normal control group. Animals treated with Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) and standard drug Metformin (40mg/kg/day p.o.) treated group showed significant reductions in CHL, LDL, VLDL, TG & significant increase in HDL level as compared with toxic control group .

Table 6: Serum Lipid Profile:

Sr. no.	Groups (n=6)	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	Triglycerides (mg/dl)
I	Control	65.15±0.82	21.82±0.86	14.00±0.35	16.32±0.65	65.49±0.75
II	Toxic control	94.32±0.70	96.32±0.48	8.82±0.29	21.15±0.29	113.32±1.46
III	Standard	68.65±0.65**	35.00±0.72**	17.82±0.46**	18.15±0.29**	75.50±0.75**
IV	Test group with Low dose of E. g. Plant leaves extract	78.15±0.59**	47.32±0.41**	13.75±0.39**	17.65±0.20**	85.75±0.98**
V	Test group with Intermediate dose of E. g. Plant leaves extract	76.82±0.59**	43.49±0.75**	14.82±0.29**	16.55±0.15**	83.69±0.83**
VI	Test group with High dose of E. g. Plant leaves extract	71.82±0.59**	37.49±0.75**	16.88±0.39**	14.45±0.13**	78.50±0.83**

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.7.3 Antioxidant Activity:

3.7.3.1 For Liver:

Diabetes mellitus significantly reduced antioxidant enzymes level of CAT, POD, SOD & GPx. After 12 days of treatment period it was observed that reductions in level of antioxidant enzymes in toxic control group as compared with normal control group. Animals treated with Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) and standard drug Metformin (40mg/kg/day p.o.) showed significant increase in level of antioxidant enzymes like CAT, POD, SOD & GPx as compared with toxic control group.

Table 7: Antioxidant Activity

Sr. no.	Groups (n=6)	CAT (kU/mg Protein)	POD (U/mg Protein)	SOD (U/mg Protein)	GPx (U/mg Protein)
I	Control	9.0±0.21	8.3±0.38	11.4±0.85	99.6±3.5
II	Toxic control	3.3±0.33	4.3±0.46	4.1±0.50	42.0±1.0
III	Standard	7.9±0.33*	7.1±0.34*	9.5±0.33*	88.0±2.0*
IV	Test group with Low dose of E. g. Plant leaves extract	5.2±0.70*	5.6±0.45*	6.8±0.32*	67.5±2.4*
V	Test group with Intermediate dose of E. g. Plant leaves extract	5.9±0.41*	6.0±0.32*	7.5±0.53*	74.0±2.2*
VI	Test group with High dose of E. g. Plant leaves extract	7.1±0.56*	6.8±0.71*	8.7±0.66*	86.2±2.7*

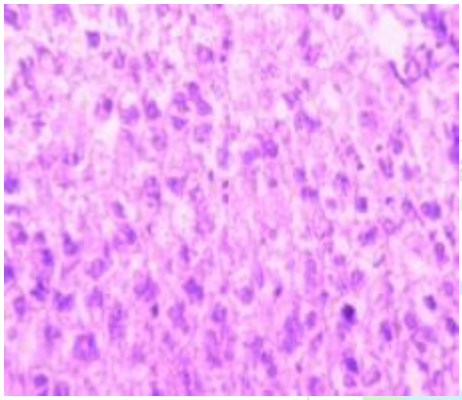
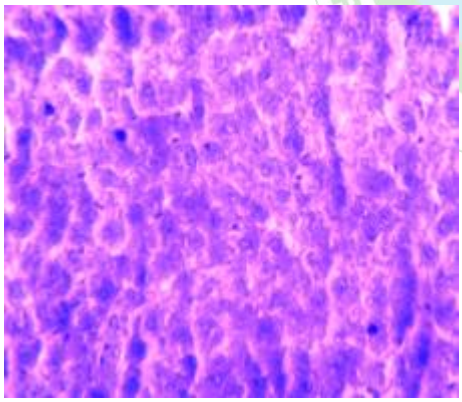
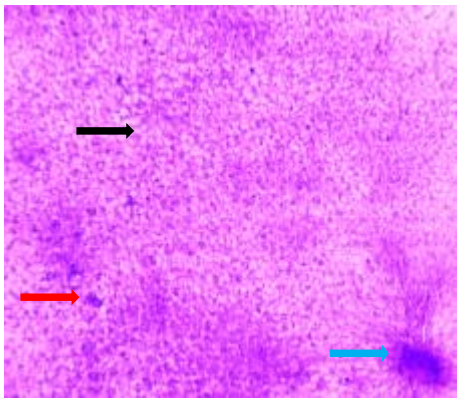
Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

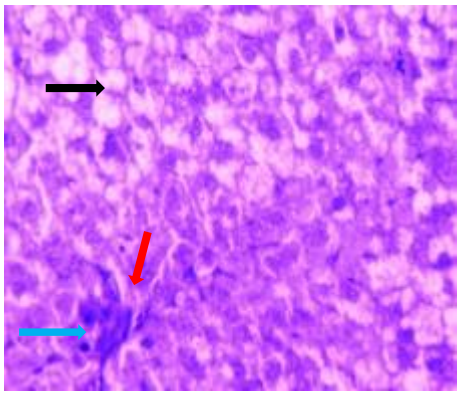
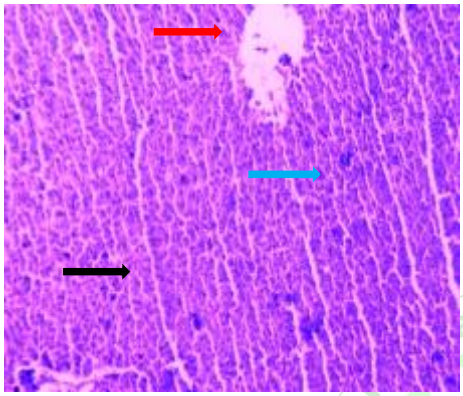
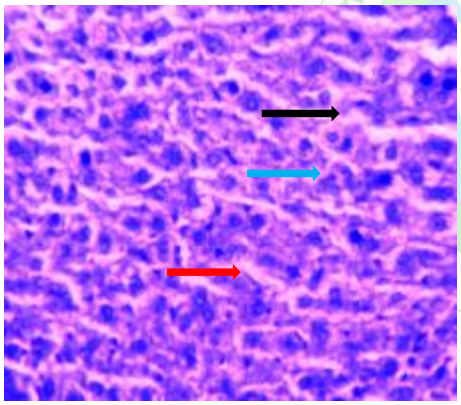
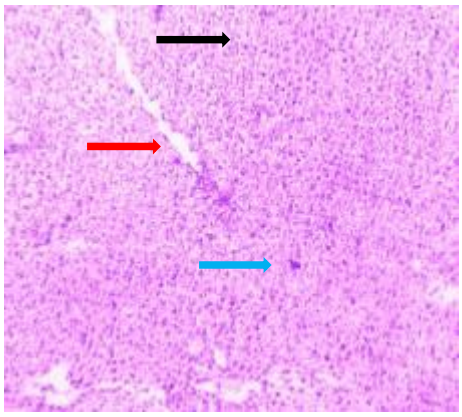
3.8 Histopathological Study:

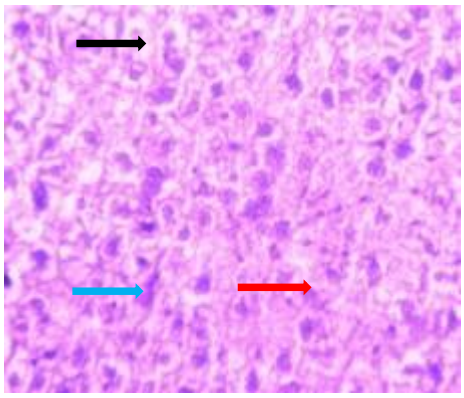
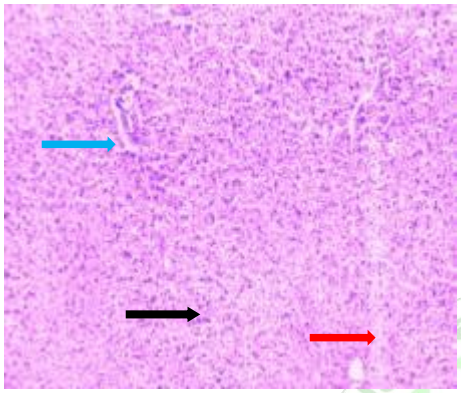
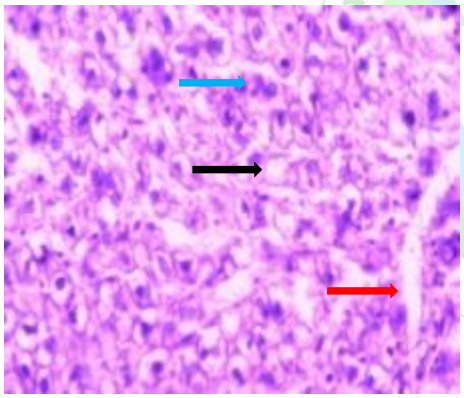
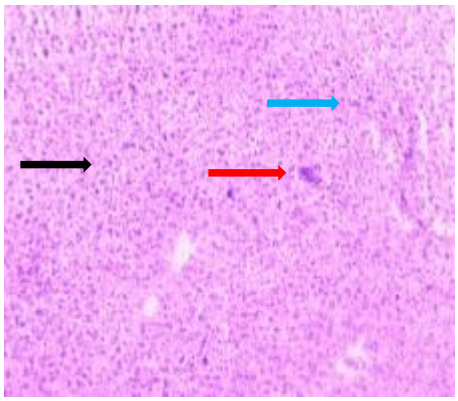
3.8.1 Liver Histopathology

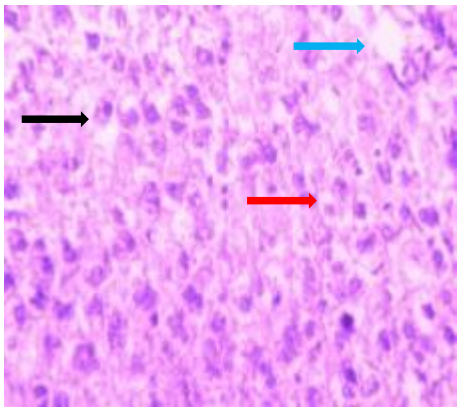
Table 8: Liver Histopathology

Sr. no.	Group	Necrosis	Cellular changes	Vascular changes
I	Control	0	0	0
II	Toxic control	+++	+++	+++
III	Standard	++	+	0
IV	Test group with Low dose of E. g. Plant leaves extract	+++	++	++
V	Test group with Intermediate dose of E. g. Plant leaves extract	++	++	+
VI	Test group with High dose of E. g. Plant leaves extract	++	+	+

	**Normal control Liver** –H & E stain 100X
	**Normal control Liver**- H & E stain 400X
	**Dexamethasone inducer group** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H&E stain 100 X

	**Dexamethasone inducer group** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 400 X
	**Standard** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 100 X
	**Standard** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H&E stain 400 X
	**Test – 1** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 100 X

	**Test – 1** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 400 X
	**Test – 2** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 100 X
	**Test – 2** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H&E stain 400 X
	**Test – 3** -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 100 X



Test – 3 -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 400 X

4. DISCUSSION

4.1 Acute toxicity, Blood glucose level & Body weight Determination:

Globally, the rapid increase the incidence of type 2 DM poses a demand for the quest of novel therapeutic drugs necessitates addition of alternative medicine. As a result number of studies has been conducted to assess the utility of herbal medicine in type 2 DM. The present study was undertaken to evaluate the Antidiabetic Potential of Methanolic Leaves Extract of *Eucalyptus globulus* Plant in Dexamethasone -Induced Diabetic Albino Wistar Rats. In the LD50 value determination, we observed that the *Eucalyptus globulus* Plant extract was safe to use in animals. There was no change in neurological, behavioural or autonomic, no lethality or toxic reactions were found with the selected doses (100, 500, 1000 and 2000mg/kg/day p.o.) until the end of study period. Therefore 200, 400 & 600mg/kg was selected for all in-vivo experiments as maximal dose.

The results of Hypoglycemic study have shown that the Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 400, 600mg/kg/day has a marked hypoglycemic potential as compared with control group (Table 1).

The Oral glucose tolerance test in normoglycemic rats, blood glucose level was significantly greater in the glucose loaded control group. Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 200, 400 and 600mg/kgp.o. and Metformin (40mg/kg i.p.) reduced the blood glucose level and improved the impaired glucose tolerance (hyperglycemia due to glucose load 2g/kg p.o.) significantly after 60 min of administration, as compared with control group (Table 2).

Induction of diabetes by Dexamethasone leads to loss of body weight due to increased muscle wasting and loss of tissue proteins, whereas body weight of animals significantly increased in Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 200, 400 and 600mg/kg/day p.o and standard drug Metformin treated group as compared with toxic control group (Table 3).

Dexamethasone is a synthetic glucocorticoid whose chronic exposure to high doses cause insulin resistance. The results of the antidiabetic study have shown a significant ($p < 0.05$) difference between the 0, 6 & 12th days fasting blood glucose levels of Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 200, 400 and 600mg/kg/day p.o. and standard drug Metformin (40mg/kg/day i.p.) treated group as compared with toxic control group (Table 4). The possible mechanism of antidiabetic action of Methanolic extract may be by increasing the pancreatic secretion of insulin from the existing beta cells, by its release from the bound form & increase in muscle glucose uptake by increased liver glucose metabolism.

4.2 Biochemical Parameters Analysis:

In Dexamethasone-induced diabetes mellitus showed improvement in biochemical parameters. Methanolic leaves extract of *Eucalyptus globulus* Plant and standard drug treated group showed significant increase in serum insulin level as compared with toxic control group (Table 5). The possible mechanism of action of Methanolic leaves extract may be by increasing the pancreatic secretion of insulin from the existing beta cells, by its release from the bound form.

The serum lipid levels are generally high in diabetes; mapping a major risk factor for coronary heart disease. Chronic exposure to Dexamethasone leads to insulin resistance promotes the increase of hormone sensitive lipase activity. Due to alteration in metabolic parameters leads to an increase in fatty acids mobilizations from adipocytes and increase in hepatic synthesis of triglycerides, which are released into the bloodstream as VLDL, LDL cholesterol. HDL cholesterols enriched with triglycerides which are rapidly hydrolysed and because of their increased catabolism, the blood level of HDL decreases. In a result of lipid profile, marked decrease in total cholesterol, LDL, VLDL and triglycerides was observed, while increase in HDL cholesterol which reduces the risk of atherosclerosis has been observed in Methanolic leaves extract & standard drug treated group which suggest that HDL is inversely related to the total body cholesterol as compared with toxic control group (Table 6). These results could thus reflect the ability of Plant extract improve the tissue sensitivity to insulin. Thus reducing the hormone sensitive lipase activity and increasing the lipoprotein lipase activity, resulting in a decrease of lipolysis these leading to hypolipidemic activity. Flavonoids have been shown to improve dyslipidemia. Thus the hypolipidemic effect of Methanolic leaves extract of *Eucalyptus globulus* Plant could be attribute to the flavonoids contained in the

Plant. Further these extract could effectively prevent cardiovascular complications related to diabetic dyslipidemia.

In antioxidants study, Diabetes mellitus reduces the antioxidant enzymes level like CAT, POD, SOD & GPx. The increase in the levels of lipid peroxidation might be indicative of a decrease in the enzymatic antioxidant defense mechanism. Animals treated with Methanolic leaves extract of *Eucalyptus globulus* Plant & standard drug treated group showed significant increase in antioxidant enzymes level like CAT, POD, SOD & GPx as compared with toxic control group (Table7).

4.3 Histopathological Examination:

In histopathological study, the fine section of Dexamethasone induced diabetic rats liver on microscopic examination using H & E,100 & 400 X stain showed the normal architecture without steatosis in the normal control group, partial loss of architecture with extensive microvascular steatosis in the toxic control group, normal architecture with microvascular steatosis in the test group i.e. Methanolic leaves extract of *Eucalyptus globulus* Plant treated group, normal architecture with focal microvascular steatosis in the test group i.e. Methanolic leaves extract of *Eucalyptus globulus* Plant treated group, normal architecture without steatosis in the Metformin treated standard group (Table 8).

The results obtained with the Methanolic leaves extract of *Eucalyptus globulus* Plant treatment in chronic diabetic model further clarified the antidiabetic potential of the extract. After 12 days of Methanolic leaves extract of *Eucalyptus globulus* Plant treatment, reductions in elevated blood glucose level,, gain in body weight, hypoglycemic activity, oral glucose tolerance, normalization in altered biochemical parameters & regeneration of damaged tissue of liver were observed, which comparable with that of the toxic control group as well as standard drug Metformin treated group. These effects could be due to the potent bioactive Polyphenol Ellagitannins present in the Plant.

5. CONCLUSION

In conclusion, it can be stated that the Methanolic leaves extract of *Eucalyptus globulus* Plant the source of Ellagitannins has beneficial effects in reducing the elevated blood glucose level as well as gained body weight, hypoglycemic activity, significant oral glucose tolerance, hepatoprotective & normalization in altered biochemical parameters of Dexamethasone-induced diabetic rats. *Eucalyptus globulus* Plant the source of Ellagitannins associated with the stimulation of insulin secretion and enhancement of muscle glucose uptake and metabolism due to regeneration in damaged tissue of liver. These effects could be due to the potent bioactive Polyphenol Ellagitannins present in the Plant. Thus justifying the claim made by ayurvedic classics. Therefore, *Eucalyptus globulus*Plant the source of Ellagitannins represents an effective antidiabetic dietary adjunct for the treatment of diabetes and a potential source for discovery of new orally active agent for future diabetes therapy.

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