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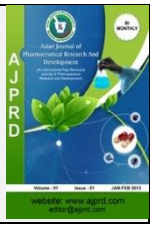

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Research Article

Evaluation of Antidiabetic Potential of *Eucalyptus Globulus* Plant Extract In Alloxan-Induced Diabetic Rats

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A B S T R A C T

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

***Method*:** Methanolic leaves extract of *Eucalyptus globulus* plant was prepared by Soxhlet extraction method and stored in refrigerator at 4°C for two days before use. Male Albino Wistar rats were made diabetic at the dose of Alloxan (150mg/kg/day i.p.). Methanolic leaves extract of *Eucalyptus globulus* plant (200mg/kg, 400mg/kg & 600mg/kg/day p.o.) was screened for antidiabetic activity. Standard drug Glibenclamide (0.5mg/kg/day i.p.) was administered to the second group of animals for 14 days. Blood glucose level and body weight of rats were recorded on Initial & Final days of treatment. Further hypoglycemic & OGTT evaluation were done. At the end of treatment biochemical estimations & histopathological examination of pancreas were also carried out.

***Result*:** The statistical data indicated, 14 Days oral administration of Methanolic leaves extract of *Eucalyptus globulus* plant caused a significant ($P< 0.05$) reductions in blood glucose level, hypoglycemicpotential, significant oral glucose tolerance &gain in body weight as compared with toxic control group. Further showed improvement in altered biochemical parameters associated with diabetes. Concurrent histopathological examination of pancreas of these animals showed regeneration by Methanolic leaves extract which were earlier necrosed by Alloxan.

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*, Alloxan, Insulin, Polyphenols.

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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, hypersensitivity, 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 Alloxan - 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:

Alloxan commonly known as Alloxan monohydrate procured from Merck/Dolphin Pharmacy Instruments Pvt. Ltd. Mumbai. Glibenclamide, (GLB) 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:

Male 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:

Male Albino Wistar Rats were made diabetic by a single intraperitoneal injection of Alloxan monohydrate (150 mg/kg/day) i.p. Alloxan monohydrate solution of 150mg/kg/day prepared in 0.9% NaCl solution and was administered within 5 minutes at a dose of 150mg/kg/day intraperitoneally. All the animals except control group were i.p. administered with Alloxan at a dose of 150mg/kg once a day for 3 days. After 72 hours of Alloxan administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with a blood glucose of 200-300mg/dl) were taken for the experiment.

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

Blood samples were drawn from tail tip of rats. Fasting blood glucose estimation and body weight measurement were done on Initial & Final day of the study. Blood glucose estimation can be done by one touch ACCU-CHECK Active Glucometer using glucose test strips. On final day, 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 withdrawn for estimation of Blood glucose level, Serum Insulin level, Lipid profile (Total cholesterol, Triglycerides, HDL, LDL, VLDL) and Hormonal estimation (Testosterone) 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°C 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 Male 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, Male 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, Male 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 Alloxan-Induced Rodent Model of Diabetes:

After 72 hours of Alloxan (150mg/kg/day i.p.) administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with a blood glucose of 200-300mg/dl) were taken for the experiment. The Male Albino Wistar rats were divided into six groups of six rats in each. All the animals were fasted overnight (14hrs.) before the treatment of test drug till 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 only vehicle that is 5% Tween 80 solution (10ml/kg/day p.o.).

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

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 globulus* 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 leavesextract 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 standard drug Glibenclamide (0.5mg/kg i.p.) 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 14 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 Glibenclamide (0.5mg/kg/day i.p.) 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)	
		Initial (1st day before treatment of test drug)	Final (Last day of treatment)
I	Control	157.67±0.77	187.33±0.88
II	Toxic control	160.17±0.71	141.00±0.78
III	Standard	160.00±0.78	170.33±1.36**
IV	Test group with Low dose of E. g. plant leaves extract	165.33±5.05	177.17±1.38**
V	Test group with Intermediate dose of E. g. plant leaves extract	160.67±0.50	175.50±1.15**
VI	Test group with High dose of E. g. plant leaves extract	163.50±1.06	171.33±1.56**

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 ofMethanolic 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 Glibenclamide (0.5mg/kg/day i.p.) 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 final (14^{th}) day post treatment .

Table 4: Effect of Methanolic Leaves Extract of *Eucalyptus globules* Plant on Fasting Blood Glucose Level in Diabetic Rats

Sr. no.	Groups (n=6)	Fasting Blood Glucose Level (mg/dl)	
		Initial (1^{st} day before treatment of test drug)	Final (Last day of treatment)
I	Control	88.51±2.08	89.17±2.03
II	Toxic control	255.01±1.19	365.68±2.84
III	Standard	255.68±1.34	117.84±1.54**
IV	Test group with Low dose of E.g. plant leaves extract	255.84±0.80	147.84±0.80**
V	Test group with Intermediate dose of E.g. plant leaves extract	256.34±2.66	135.68±2.26**
VI	Test group with High dose of E. g. leaves plant extract	257.40±2.66	127.68±2.02**

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 & Hormonal Estimation

After 14 days of treatment period it was observed that decreased serum insulin level & testosterone 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 Glibenclamide (0.5mg/kg/day i.p.) treated group showed a significant increase in the serum insulin & testosterone level as compared with toxic control group.

Table 5: Serum Insulin Level & Hormonal Estimation

Sr. no.	Groups (n=6)	Serum Insulin Level *(µU/ml)*	Testosterone Level (ng/ml)
I	Control	18.18±0.56	9.88±0.64
II	Toxic control	7.15±0.32	3.95±0.53
III	Standard	16.68±0.36**	8.49±0.83*
IV	Test group with Low dose of E. g. plant leaves extract	12.35±0.34**	5.32±0.48*
V	Test group with Intermediate dose of E. g. plant leaves extract	13.35±0.38**	6.54±0.65*
VI	Test group with High dose of E. g. plant leaves extract	15.35±0.34**	8.33±0.73*

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 14days of treatment period it was observed that increased in 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 Glibenclamide (0.5mg/kg/day i.p.) 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	67.17±0.84	23.84±0.88	16.01±0.37	18.34±0.67	67.51±0.77
II	Toxic control	96.34±0.72	98.34±0.50	10.84±0.31	23.17±0.31	115.34±1.48
III	Standard	70.67±0.67**	37.01±0.74**	18.84±0.48**	19.17±0.31**	77.51±0.77**
IV	Test group with Low dose of E. g. plant leaves extract	78.17±0.61**	49.34±0.43**	14.75±0.41**	18.67±0.22**	89.55±0.99**
V	Test group with Intermediate dose of E. g.	75.84±0.61**	45.51±078**	15.84±0.31**	17.17±0.17**	85.51±0.85**
VI	Test group with High dose of E. g. plant leaves extract	71.80±0.55**	39.50±076**	17.86±0.33**	15.17±0.19**	79.58±0.65**

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).

3.7.3Antioxidant Activity:

3.7.3.1 For Pancreas

Diabetes mellitus significantly reduced antioxidant enzymes level of CAT, POD, SOD & GPx. After 14 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 Glibenclamide (0.5mg/kg/day i.p.) showed significant increase in level of antioxidant enzymes like CAT, POD, SOD & GPx as compared with toxic controlgroup.

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	7.9±0.73	6.5±0.50	6.8±0.76	84.4±2.0
II	Toxic control	2.6±0.22	2.0±0.25	1.8±0.16	40.0±1.5
III	Standard	6.5±0.45*	5.7±0.30*	5.9±0.20*	75.6±1.4*
IV	Test group with Low dose of E. g. plant leaves extract	3.8±0.30*	3.5±0.42*	4.0±0.11*	56.0±2.5*
V	Test group with Intermediate dose of E. g. plant leaves extract	4.5±0.42*	3.9±0.25*	4.5±0.40*	61.0±2.7*
VI	Test group with High dose of E. g. plant leaves extract	5.8±0.68*	5.5±0.33*	5.7±0.15*	72.0±2.4*

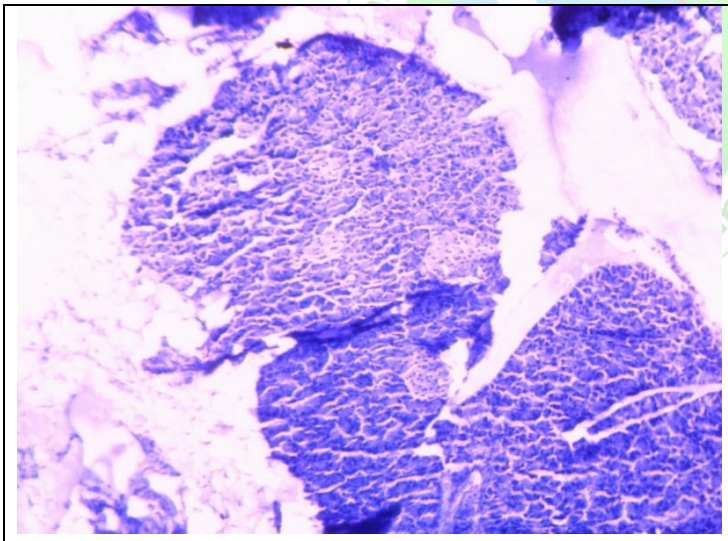
Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.8 Histopathological Study:

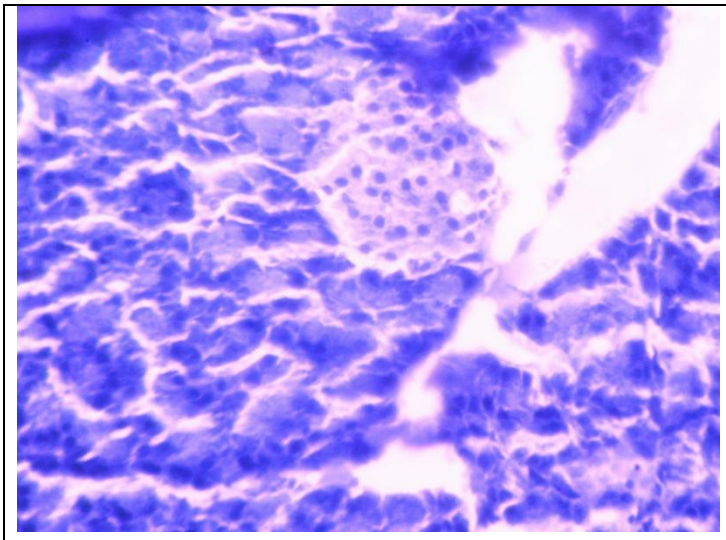
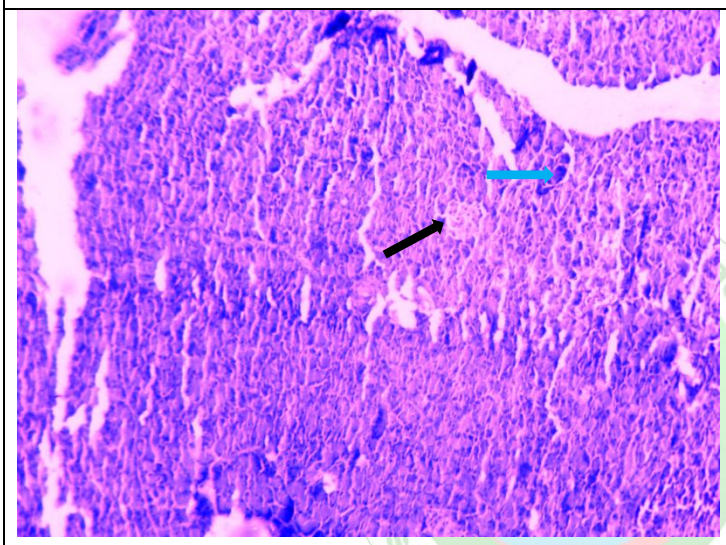
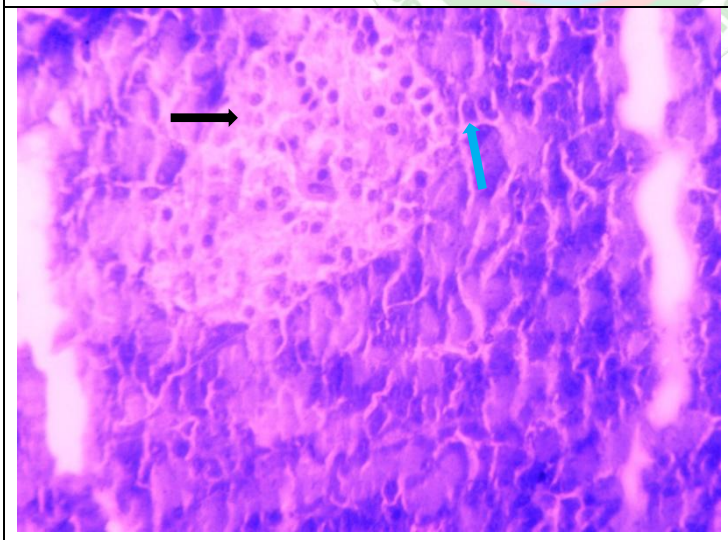
3.8.1 Pancreas Histopathology

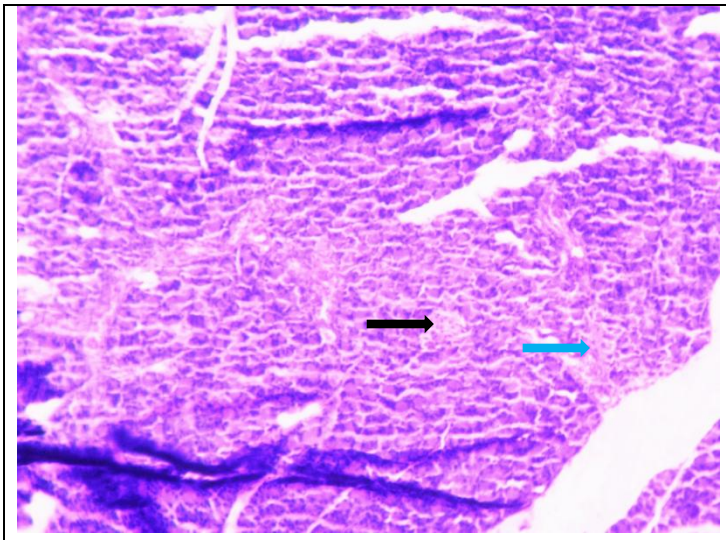
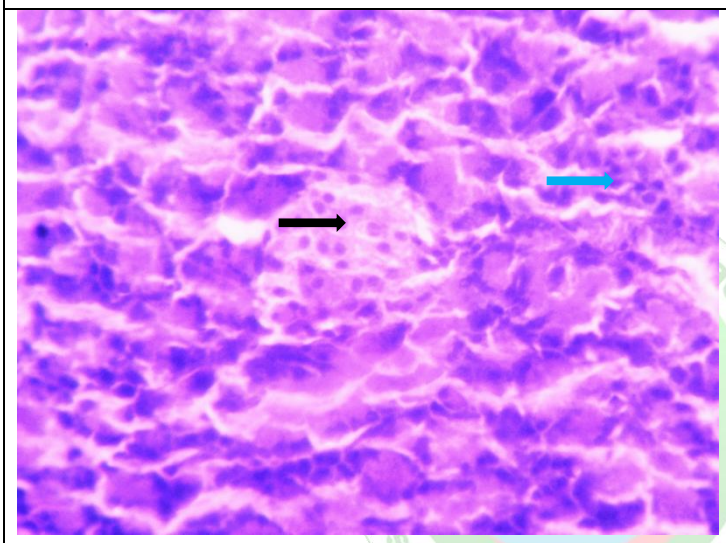
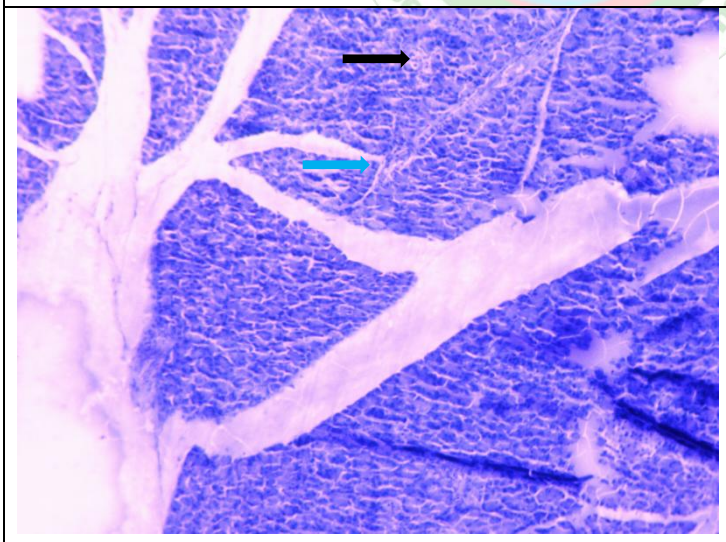
Table 8: Pancreas Histopathology

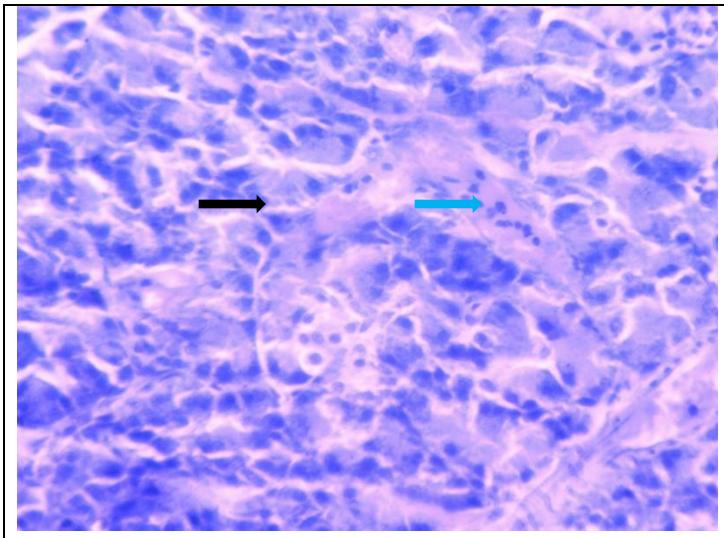
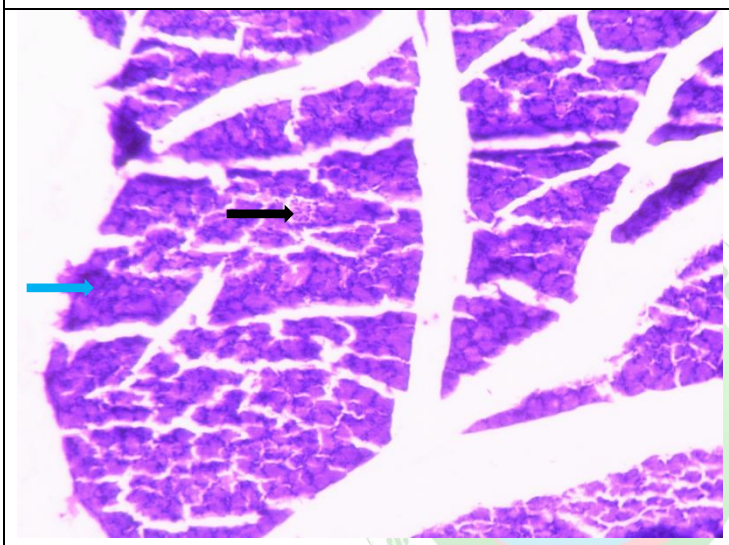
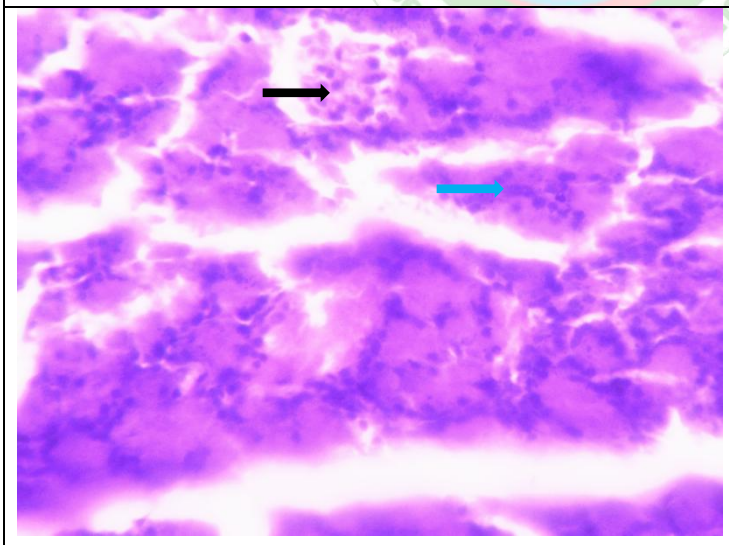
Sr. no.	Group	Necrosis of islets	Cellular changes
I	Control	0	0
II	Toxic control	+++	+++
III	Standard	++	+
IV	Test group with Low dose of E. g. plant leaves extract	+++	++
V	Test group with Intermediate dose of E. g. plant leaves extract	++	++
VI	Test group with High dose of E. g. plant leaves extract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

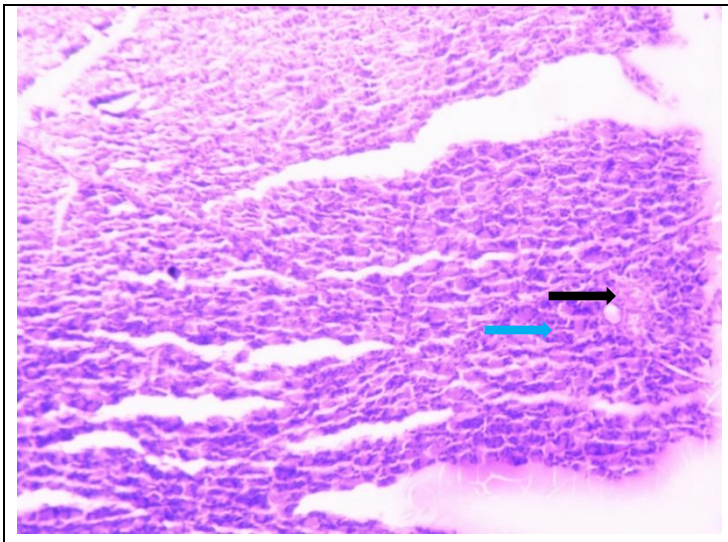


Normal control Pancreas- H & E stain 100X

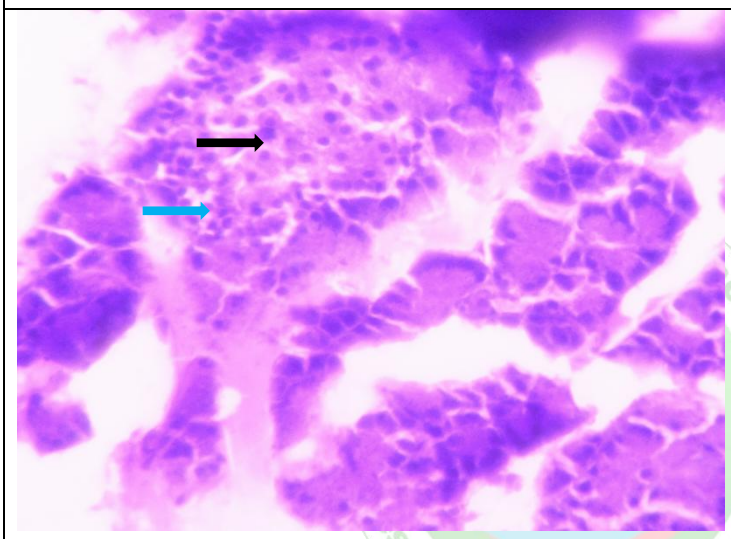
	**Normal control Pancreas-** H & E stain 400X
	**Alloxan inducer group** -Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 100X
	**Alloxan inducer group** -Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 400X

	**Standard**-Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 100X
	**Standard**-Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 400X
	**Test – 1**-Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 100X

	**Test – 1**-Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 400X
	**Test – 2**-Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 100X
	**Test – 2**-Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 400X



Test – 3-Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 100X



Test – 3-Photograph showing necrosis of islets of Langerhans (black arrow), cellular infiltration (blue arrow) H & E stain 400X

4. DISCUSSION

4.1 Acute Toxicity, Blood Glucose Level & Body Weight Determination:

Globally, the rapid increase the incidence of type 2 DMposes 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 globules* Plant in Alloxan-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/kg p.o.and Glibenclamide (0.5mg/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 Alloxan 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 Glibenclamide (0.5mg/kg/day i.p.) treated group as compared with toxic control group (Table 3).

Alloxan has two distinct pathological effects: Alloxan is a toxic glucose analogue it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induce ROS formation leading to demolition of pancreas β-cells & selective necrosis leading to hypoinsulinemia and hyperglycemia. The results of the

antidiabetic study have shown a significant ($p < 0.05$) difference between the initial and final 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 Glibenclamide (0.5mg/kg/day i.p.) treated groupsas 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 Alloxan-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.

Diabetes causes hypogonadism through multiple mechanisms. The pathogenesis of low testosterone in animal models with diabetes includes impaired hypothalamic signaling and hypogonadotropic hypogonadism as well as reduced testosterone production by testicular Leydig cells. In hormonal estimation, animals treated with Methanolic leaves extract of *Eucalyptus globulus* plant & standard drug treated group showed significant increase in testosterone level as compared with toxic control group (Table 5).

The serum lipid levels are generally high in diabetes; mapping a major risk factor for coronary heart disease. Chronic exposure to Alloxan leads to destruction in ß cells of pancreas and 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 *Eucalyptus globulus* plant and 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 significantly reduced antioxidant enzymes level CAT, POD, SOD, GPx levels. 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 showed significant increase in antioxidant enzymes level CAT, POD, SOD & GPx as compared with toxic control rats (Table 7).

4.3 Histopathological Examination:

In histopathological study, the fine section of Alloxan-induced diabetic rats pancreas on microscopic examination using H&E stain, 100 & 400X showed the presence of islets of Langerhans, blood vessels, connective tissues, inter and arrangement of islets of Langerhans was normal with tightly arranged cells and uneven distribution throughout the lob-necrosis. The interlobular and intralobular duct showed widening. In standard Glibenclamide and Methanolic leaves extract of *Eucalyptus globulus* plant treated groups it was observed that although the gap between the islets was more than lesser number of islets as compared to vehicle control group, it was significantly much better than the toxic control group. The dose of Methanolic leaves extract of *Eucalyptus globulus* plant at the dose (200, 400 and 600mg/kg/day) had immensely protected and regenerated the cells (Table 8). Thus the histopathological examination revealed good protective and regenerative property of this herbal extract.

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 14 days of Methanolic leaves extract of *Eucalyptus globulus* plant treatment, reductions in elevated blood glucose level, gain in body weight, hypoglycemic potential, oral glucose tolerance, normalization in altered biochemical parameters & regeneration in damaged tissue of pancreas were observed, which comparable with that of the toxic control group as well as standard drug Glibenclamide 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, hypoglycemicpotential, significant oral glucose tolerance& normalization in altered biochemical parameters of Alloxan-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 pancreas. 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 globules* 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.

6. REFERENCES

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