International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

Evaluation of Antidiabetic Potential of Eucalyptusglobulus Plant Extract in Diabetic Rats

Sul Komal*[1], Ujwala Kamble*[2]
[1]*Department of Pharmacology, Shriram Shikshan Sanstha's College of Pharmacy, Paniv, India.*

Date of Submission: 01-12-2024 Date of Acceptance: 10-12-2024

ABSTRACT:
EvaluationofAntidiabeticPotentialofEucalyptusglobulesPlantExtractinDiabeticRats.
Method: MethanolicleavesextractofEucalyptus globulus plant was prepared by Soxhlet extraction method and stored in refrigerator at 4℃ fortwodaysbeforeuse.MaleAlbinoWistarratswe remade diabetic at the dose of Alloxan (150mg/kg/day i.p.). Methanolic leaves extract ofEucalyptusglobulusplant(200mg/kg,400mg/kg&600mg/kg/dayp.o.)wasscreenedforantidiabeticactivity.StanderddrugGlibenclamide(0.5mg/kg/dayi.p.)was administered tothe secondgroupofanimalsfor14 days.Blood glucose level and body weight of rats were recorded onInitial&Finaldaysoftreatment.Furtherhypoglycemic&OGTTevaluationweredone.Attheendoftreatmentbiochemicalestimations&histopathologicalexaminationofpancreaswerealsocarriedout.
Result: Thestatisticaldataindicated, 14 Days oral administration of Methanolic leaves extract of Eucalyptus globulus plant caused a significant($P<$ 0.05) reductionsinbloodglucoselevel,hypoglycemicpotential, significantoral glucosetolerance&gain inbodyweight as comparedwith toxiccontrolgroup.Furthershowedimprovement inalteredbiochemicalparametersassociatedwith diabetes.ConcurrenthistopathologicalexaminationofpancreasoftheseanimalsshowedregenerationbyethanolicleavesextractwhichwereearliernecrosedbyAlloxan.
Conclusion: ResultsobtainedinthisstudysubstantiatetheAntidiabetic potential of Methanolic leaves extract of Eucalyptus globulus plant thesourceofEllagitanninsabioactivepolyphenol andcouldbeconsideredforfurtherevaluationinclinicalstudiesanddrugdevelopment.
Keywords: Antidiabeticpotential,OGTT,Eucalyptusglobulus,Alloxan,Insulin,Polyphenols.

I. INTRODUCTION :

Diabetesmellitusisthechronicmetabolic&pancreaticisletdisordermainlycharacterizedbydisruptionincarbohydrates, protein, and fatmetabolismcausedbyaninabilitytoproduceinsulinoradefectinutilization.Thehyperglycemiacausedduetodecreasedinsulinproductioniscalled Type-1diabetesandhyperglycemiadueinsufficientinsulinutilizationiscalled Type-2diabetes.Thefeatureofdiabetesmellitusispolyuria,polydipsia,weightgainandpolyphagia.Itisalsocharacterizedbychronichyperglycemiaandglucosuria,caused by anabsoluteorrelativedeficiencyofinsulin.Thismayresultinthedevelopmentoffurthercomplicationswhich include hypertension, atherosclerosis,ketosis,gangreneandmicrocirculatorydisorders.Itisalsoassociatedwithlong-termcomplicationsincludingretinopathy,nephropathy,neuropathyandangiopathy[1].TheIDF(International Diabetesfederation)hassubsequently released estimates of the numbers of peoplewithdiabetesfor2003andforecastsfor2025of194million and 334 million, respectively. India leads the worldwith largest number of diabetic subjects earning of term"diabetes capital of theworld"[2]. Hyperglycemia can behandedinitiallywithoralsynthetic2AdvancesinPharmacologicalSciencesagentandinsulintherapy.Glucose lowering drugs usually succeed in lowering bloodsugar levels, therapeutic agents like Insulin, Sulfonylureas,Meglitinides,Biguanides,Thiozolidinediones,DPP-4 inhibitors, α-Glucosidase Inhibitors, Incretin agonists, D2Agonist may reduce the risk of type 2 diabetes but healthylifestyle choices remain essential[3]. However, on chronicusage most of these agents produced several side effectsincludinghypoglycemiccoma,insulinresistance,hyper-sensitivity, jaundice, abdominal pain, anorexia and metallictaste. Because of the high mortality and morbidity arisingfrom its attendant complications and problems associatedwith the use of conventional antidiabetic agents[4]. In thenatural

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

system of medicine, many plants have been claimedtobeusefulforthetreatmentofdiabetesmellitus.Thedependenceoflargeruralpopulationonmedicinalplantsfor treatment of diabetes is because of its availability andaffordability.Thecurrentworldwidetrendstowardsutilizationofplant-derivednaturalremedieshave,therefore, created a dire need for accurate and up-to-dateinformation on the properties, uses, efficacy, safety, quality&lesscostofmedicinalplantproductsthanthesemi-synthetics or synthetics. The plant kingdom has become atarget for the search by multinational drug and biologicallyactiveleadcompounds.Inthisregardherbal,ayurvedicremediescanimprovediabeticconditions withoutsideeffects[5].Ellagitannins(ETs)andellagica cid(EA)arepolyphenols present in some fruits, nuts and seeds, such aspomegranates, black raspberries, raspberries, strawberries,walnuts, almonds & also present in „Eucalyptus globulusplant". Ellagitanninscontainvariousnumbers ofhexahydroxydiphenoyl units, as well as galloyl units and/orsanguisorboyl units bounded to sugar moiety. In order todeterminethequantityofeveryindividualunit,thehydrolysisoftheextractswithtrifluoroaceticacidinmethanol/water system is performed.They form a diversegroupofbioactivepolyphenolswithanti-inflammatory,anticancer,antioxidantandantimicrobial(antibacterial,antifungal and antiviral) activity[6]. So,thepresent studywasundertakentoEvaluateAntidiabeticPotentialofEucalyptusglobulusplantExtractthesourceofEllagitanninsin Alloxan-induced Diabetic Rats.

II. MATERIALS&METHODS

CollectionofPlantMaterial:

Eucalyptus globulus plant was collected from Local area ofKolhapur District, Maharashtra, India in January 2021 andauthenticated as Eucalyptus globulus (Family: Myrtaceae)by Department of Botany, Yashwantrao Chavan College ofScience,Satara,Maharashtra,Indiabasedonthetaxonomical features of the whole plant material includingLeaves.

PreparationofMethanolicExtract:

Leaves of Eucalyptus globulus plant were shade dried forone week after proper cleaning. Eucalyptus globulus plantleaves were coarsely grounded & Methanolic leaves extractwas prepared using Soxhlet apparatus by hot percolationmethod. The obtained extract was concentrated to drynessusing rotatory evaporator underreducedpressure & lowpressure(<40℃).Extractwas kept in air-tight containerandstoredat4℃ forfurtherstudies.

PhytochemicalScreening:

The extract was subjected to phytochemical analysis to testthepresenceofvolatileoils,carbohydrates,alkaloids,glycosides,polyphenols,flavonoids,tannins,propanoids,sterolsterpenoids,ketones&alcoholsintheleaves extract.

DrugsandChemicals:

AlloxancommonlyknownasAlloxanmonohydrateprocured from Merck/Dolphin Pharmacy Instruments Pvt.Ltd.Mumbai.Glibenclamide,(GLB)obtainedfromAventisPharma,Ltd.Goa.ACCU-CHECKActiveGlucometerprocuredfromRocheDiabetesCare,India/DolphinPharmacyInstrumentsPvt.Ltd.Mumbai.

Animals&HousingCondition:

Male Albino Wistar Rats of (150-200gm) were selected forexperimentalstudy.Theanimalsweremaintainedunderstandard laboratory conditions in an animal house approvedby Committeefor the Purpose of Control and Supervisionof Experiments on Animals (CPCSEA). The animals werekeptandmaintainedunderlaboratoryconditionsoftemperature 22 ± 2℃, relative humidity 50 ± 15% and 12hrs. light/dark cycle. They were allowed free access to food(standardpellets)andwateradlibitum.Experimentalprotocols andprocedures usedinthisstudy was approvedby the Institutional Animal Ethics Committee (IAEC) ofYSPM"s, YTC, Faculty of Pharmacy, Satara, Maharashtra,India.

InductionofDiabetes:

Male Albino Wistar Rats were made diabetic by a singleintraperitonealinjectionofAlloxanmonohydrate(150 mg/kg/day)i.p.Alloxanmonohydratesolutionof150mg/kg/daypreparedin0.9%NaClsolutionandwasadministered within 5 minutes at a dose of 150mg/kg/dayintraperitoneally.Allthe animalsexceptcontrolgroupwerei.p.administeredwithAlloxanatadoseof150mg/kgoncea day for 3 days. After 72 hours of Alloxan administration,ratswithmoderatediabeteshavingglyc

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

osuriaandhyperglycemia (i.e., with a blood glucose of 200-300mg/dl)weretakenfor the experiment.

\

Collection of Blood Samples, Blood Glucose & BodyWeightDetermination:

Blood samples were drawn from tail tip of rats. FastingbloodglucoseestimationandbodyweightmeasurementweredoneonInitial&Finaldayofthestudy.Bloodglucoseestimationcanbedonebyonetouch ACCU-CHECK Active Glucometer using glucose test strips. Onfinalday,bloodwascollectedfromretro-orbitalplexusunder mild ether anesthesia from overnight fasted rats andfasting bloodsugarwas estimated. After that body weightofanimalswasdetermined.

BiochemicalEstimation:

Bloodsampleswerewithdrawnforestimationofbloodglucoselevel,SerumInsulinlevel,Lipidprofile(Totalcholesterol,Triglycerides,HDL,LDL,VLDL)andHormonal estimation (Testosterone) in the serum sample.Aftertheendofrespectivetreatment,animalswaresacrificed with high dose of anaesthesia and the tests organswereremoved,weighedandstoredat-20°CforfurtherAntioxidantandHistopathologicalstudies.

1.1 ExperimentalDesign:

1.1.1 AcuteToxicityStudy:

AcutetoxicitystudywascarriedoutfortheEucalyptusglobulus plant by adapting fixed dose method of CPCSEA,OECD guidelines no. 423. Healthy Male Albino Wistar ratswere randomly divided into 4 groups with 3 animals in eachgroup. The animals were kept fasted overnight providingonly water, after which the Methanolic leaves extract ofEucalyptus globulusplantwere administeredorally withincreasing doses (100, 500, 1000 and 2000mg/kg/day) byintra gastric tube to determine the safe doses by up anddownstaircasemethod.Theanimalswereobserved continuously for 1 hr., then frequently for 4 hrs. and later atthe end of 24hrs.Forgeneral neurological &behaviouralor autonomic profile. Further, one group was administeredhigh dose of Eucalyptus globulus plant leaves extract orallyonce a day for 15 days and observed for any lethality anddeath.

1.1.2 HypoglycemicEvaluation:

ForHypoglycemicevaluation,MaleAlbino WistarRatswere used and divided into four groups of six animals ineach group. Animals were kept fasted overnight (14hrs.)beforetreatment.

GroupI- (Control)ratsreceivedvehiclethatwas5%Tween80(10ml/kg/dayp.o.).

GroupII- (Test1)ratsreceivedMethanolicleavesextractofEucalyptusglobulusplant(200mg/kg/dayp.o.)solubilized in5%Tween80solution.

Group III- (Test2) rats received Methanolic leaves extractofEucalyptusglobulusplant(400mg/kg/dayp.o.)solubilized in5%Tween80 solution.

Group IV- (Test3) rats received Methanolic leaves extractofEucalyptusglobulusplant(600mg/kg/dayp.o.)solubilized in5%Tween80solution.

Blood glucose was estimated on 0, 1, 2, 3 & 4th day of thetreatmentusingtheACCU-CHECK ActiveGlucometer.

1.1.3 OralGlucoseToleranceTest:

For OGTT evaluation, Male Albino Wistar Rats were usedand divided into five groups of six animals in each group.Animalswerekeptfastedovernight(14hrs.)beforetreatment.

GroupI-(Control)ratsreceivedvehiclethatwasD-Glucose(2gm/kgp.o.).

GroupII- (Standard)ratsreceivedGlibenclamide(0.5mg/kgi.p.).

Group III- (Test1) rats received Methanolic leaves extractof Eucalyptus globulus plant (200mg/kg p.o.) solubilized in5%Tween80solution.

Group IV- (Test2) rats received Methanolic leaves extractof Eucalyptus globulus plant (400mg/kg p.o.) solubilized in5%Tween80solution.

Group V- (Test3) rats receivedMethanolic leavesextractof Eucalyptus globulus plant (600mg/kg p.o.) solubilized in5%Tween80solution.

D-glucose (2gm/kg p.o.) was administered to all the ratsafteronehourofadministrationofdifferenttreatments.Blood glucose was estimated at 30, 60, 90 & 120 min afterD-GlucosetreatmentusingtheACCU-CHECKActiveGlucometer.

1.1.1 Alloxan-InducedRodentModelofDiabetes:

After72hoursofAlloxan(150mg/kg/dayi.p.) administration,ratswithmoderatediabeteshavingglycosuria and hyperglycemia (i.e., with a blood glucose of200-300mg/dl) were taken for the experiment. The MaleAlbino Wistar ratswere dividedinto six groups of six

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

ratsineach.Alltheanimalswerefastedovernight(14hrs .)beforethetreatmentoftestdrugtillend ofstudy.

GroupI- (Control)ratsreceivedvehiclethatwas5%Tween80solution(10ml/kg/dayp.o.).

Group II- (Toxic Control) rats received only vehicle that is5%Tween80solution(10ml/kg/dayp.o.).

GroupIII- (Standard)ratsreceivedGlibenclamide(0.5mg/kg/dayi.p.).

Group IV- (Test1) rats received Methanolic leaves extractofEucalyptusglobulusplant(200mg/kg/dayp.o.)solubilized in5%Tween80solution.

Group V- (Test2) rats receivedMethanolic leavesextractofEucalyptusglobulusplant(400mg/kg/dayp.o.)solubilized in5%Tween80solution.

Group VI- (Test3) rats received Methanolic leaves extractofEucalyptusglobulusplant(600mg/kg/dayp.o.)solubilized in5%Tween80solution.

1.2 StatisticalAnalysis:

All values of results were presented as mean ± standarderror of mean (SEM). The statistical analysis involving twogroups was evaluated by means of Student˝st-test, whereasonewayanalysisofvariance(ANOVA)followedbyDunnett˝smultiplecomparisonposttestwasused forstatistical comparison between control and various treatedgroups.Statisticalsignificancewasacceptedatthep< 0.05values.

III. RESULTS

QuantitativePhytochemicalTest:

Theyieldofextractwasfoundtobe4.8%.Thephytochemicalanalysisrevealedthattheethanolicleavesextract ofEucalyptusglobulusplantcontainsasignificant amount of volatile oils, carbohydrates, alkaloids,glycosides,polyphenols,flavonoids,tannins,propanoids,sterolsandterpenoids,ketones, alcohols.

AcuteToxicityStudy:

IntheLD50valuedetermination,weobserved thattheEucalyptusglobulusplantextractwassafetouse inanimals. There was no change in neurological, behaviouralor autonomic, no lethality or toxic reactions were foundwith the selected doses (100, 500, 1000 and 2000mg/kg/dayp.o.) until the end of study period. Therefore 200, 400 &600mg/kgwasselectedforallinvivoexperimentsas maximaldose.

HypoglycemicEffectofMethanolicLeavesExtract of Eucalyptusglobulus PlantinNormalRats:

Theresultsfromthestudyclearlyindicatedthatthe administration of Methanolic leaves extract of Eucalyptusglobulus plant at the dose 200, 400 and 600mg/kg/day p.o.reduced the blood glucose level significantly on 4th day ascomparedwithnormalcontrolgroup.

Table1: HypoglycemicEffectofMethanolicLeavesExtractofEucalyptusglobulusPlantinNormalRats

Sr.no.	Groups(n=6)	FastingBloodGlucoseLevel(mg/dl)				
		0thday	1stday	2ndday	3rdday	4thday
I	Control	86.00±0.73	85.16±0.65	83.50±1.05	82.83±1.07	82.33±0.49
II	TestgroupwithLowdose ofE.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	TestgroupwithIntermediatedoseof E.g.plantleavesextract	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).

Effect of Methanolic Leaves Extract of Eucalyptusglobulus Plant on the Oral Glucose Tolerance Test inNormalRats:

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

Theresultsfromthestudyclearlyindicatedthattheadministrationof Methanolicleavesextractof Eucalyptus globulus plant at the dose 200, 400 and 600mg/kg p.o. andstandard drug Glibenclamide (0.5mg/kg i.p.) reduced thebloodglucoselevel(hyperglycemiaduetoglucoseload2g/kg p.o.) significantly after 60 min of administration, ascomparedwithcontrolgroup.

Table2:EffectofMethanolicLeaves ExtractofEucalyptusglobulusPlantontheOralGlucoseToleranceTestinNormalRats

Sr.no.	**Groups(n=6)**	**FastingBloodGlucoseLevel(mg/dl)inmin**				
		0min	**30min**	**60min**	**90min**	**120min**
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	TestgroupwithLowdoseof E.g.plant leavesextract	94.17±0.60	96.67±1.05	90.00±0.58	89.34±0.33	88.51±0.42*
IV	TestgroupwithIntermediatedoseofE.g. plantleavesextract	94.84±0.79	95.87±1.01	90.67±0.91	89.84±0.70	88.63±0.42*
V	TestgroupwithHighdoseof E.g.plant leavesextract	93.66±1.05	95.74±1.01	89.84±0.70	88.63±0.42	85.94±0.95*

Valuesaremean± SEM,n=6,whencompared withnormalbyusingonewayANOVAfollowed byDunnette'smultiplecomparisontest.(*p<0.05,**p <0.01).

Effect of MethanolicLeavesExtractofEucalyptusglobulus PlantonBodyWeight ofDiabetic Rats:

Attheendof14daystreatment,bodyweightwassignificantlydecreasedintoxiccontrolgroupascomparedwithnormalcontrolgroup&significantlyincreasedinMethanolic leaves extract of Eucalyptus globulus plant atthe dose (200, 400 and 600mg/kg/day p.o.) and standarddrug Glibenclamide(0.5mg/kg/day i.p.)treated groupascomparedwithtoxic controlgroup

Table3: EffectofMethanolicLeavesExtractofEucalyptusglobulusPlantonBodyWeightofDiabeticRats

Sr.no.	**Groups(n=6)**	**BodyWeightofAnimals(gm)**	
		Initial(1st daybeforetreatmentoftestdrug)	**Final(Lastdayoftreatment)**
I	Control	157.67±0.77	187.33±0.88
II	Toxiccontrol	160.17±0.71	141.00±0.78
III	Standard	160.00±0.78	170.33±1.36**
IV	TestgroupwithLowdoseofE.g.plant leavesextract	165.33±5.05	177.17±1.38**
V	TestgroupwithIntermediatedoseofE.g. plantleavesextract	160.67±0.50	175.50±1.15**
VI	TestgroupwithHighdoseofE.g.plant leavesextract	163.50±1.06	171.33±1.56**

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

Valuesare mean±SEM,n=6,whencomparedwithnormal byusing oneway ANOVAfollowedbyDunnette'smultiplecomparisontest.(*p<0.05,**p <0.01).

Effect ofMethanolicLeavesExtractofEucalyptusglobulusPlantonFastingBloodGlucoseLevelinDiabeticRats:

A marked rise in fasting blood glucose level was observedin toxic control group as comparedwith normal controlgroup.TheMethanolicleavesextractofEucalyptus globulusplantandstandarddrugGlibenclamide(0.5mg/kg/dayi.p.)treatedgroupwhichproducedasignificant reduction in blood glucose level as comparedwithtoxiccontrolgroup.Methanolicleavesextract ofEucalyptusglobulusplantatthedose(200,400 and600mg/kg/day p.o.) exhibited a dose dependent significantantidiabeticpotentialonfinal(14th)daypost treatment

Table4: EffectofMethanolicLeavesExtractofEucalyptusglobulesPlantonFastingBloodGlucoseLevelinDiabeticRat

Sr.no.	Groups(n=6)	FastingBloodGlucoseLevel(mg/dl)	
		Initial(1st daybeforetreatmentoftestdrug)	Final(Lastdayoftreatment)
I	Control	88.51±2.08	89.17±2.03
II	Toxiccontrol	255.01±1.19	365.68±2.84
III	Standard	255.68±1.34	117.84±1.54**
IV	TestgroupwithLowdoseofE.g.plant leavesextract	255.84±0.80	147.84±0.80**
V	TestgroupwithIntermediatedoseofE.g. plantleavesextract	256.34±2.66	135.68±2.26**
VI	TestgroupwithHighdoseofE.g.leaves plantextract	257.40±2.66	127.68±2.02**

Valuesare mean±SEM,n=6,whencomparedwithnormal byusing oneway ANOVAfollowedbyDunnette'smultiplecomparisontest.(*p<0.05,**p <0.01).

Effect of Methanolic Leaves Extract OfWucalyptus globulus plant on Biochemical Parameters in Diabetic Rats: SerumInsulinLevel&HormonalEstimation

After14daysoftreatmentperioditwasobservedthatdecreasedseruminsulinlevel&testosteroneintoxic controlgroupascomparedwithnormalcontrolgroup.AnimalstreatedwithMethanolicleavesextractofEucalyptusglobulusplantatthedose(200,400and600mg/kg/dayp.o.)andstandarddrugGlibenclamide(0.5mg/kg/dayi.p.)treatedgroupshowedasignificantincreaseinthes eruminsulin&testosteronelevelascomparedwithtoxic controlgroup.

Table5: SerumInsulinLevel&HormonalEstimation

Sr.no.	Groups(n=6)	SerumInsulinLevel(µU/ml)	TestosteroneLevel(ng/ml)
I	Control	18.18±0.56	9.88±0.64
II	Toxiccontrol	7.15±0.32	3.95±0.53
III	Standard	16.68±0.36**	8.49±0.83*
IV	TestgroupwithLowdoseofE.g.plantleavesextract	12.35±0.34**	5.32±0.48*
V	TestgroupwithIntermediatedoseofE.g.plantleavesextract	13.35±0.38**	6.54±0.65*
VI	TestgroupwithHighdoseofE.g.plantleavesextract	15.35±0.34**	8.33±0.73*

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

Valuesaremean±SEM, n=6,whencomparedwithnormalbyusingonewayANOVA followedbyDunnette'smultiplecomparisontest.(*p<0.05,**p<0.01).

SerumLipidProfile:

After14daysoftreatmentperioditwasobservedthatincreased in level of CHL, LDL, VLDL, TG & decreasedHDL level in toxic control group as compared with normalcontrolgroup.AnimalstreatedwithMethanolic leaves extract of Eucalyptus globulus plant at the dose (200, 400and 600mg/kg/day p.o.) and standard drug Glibenclamide(0.5mg/kg/dayi.p.)treatedgroupshowedsignificantreductions in CHL, LDL, VLDL, TG & significant increaseinHDLlevelascomparedwithtoxiccontrolgroup.

Table6: SerumLipidProfile

Sr. no.	Groups(n=6)	TotalCholesterol(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	Toxiccontrol	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	TestgroupwithLowdose ofE.g.plantleavesextract	78.17±0.61**	49.34±0.43**	14.75±0.41**	18.67±0.22**	89.55±0.99**
V	Testgroupwith Intermediatedoseof E.g.	75.84±0.61**	45.51±078**	15.84±0.31**	17.17±0.17**	85.51±0.85**
VI	TestgroupwithHighdose ofE.g.plant leavesextract	71.80±0.55**	39.50±076**	17.86±0.33**	15.17±0.19**	79.58±0.65**

Valuesaremean±SEM, n=6, whencomparedwithnormalbyusingonewayANOVA followedbyDunnette'smultiple comparisontest. (*p<0.05,**p< 0.01).

AntioxidantActivity:
ForPancreas

Diabetesmellitussignificantlyreducedantioxidantenzymeslevelof CAT,POD,SOD&GPx.After14daysoftreatment period it was observed that reductions in levelofantioxidantenzymesintoxiccontrolgroupascomparedwithnormalcontrolgroup.Animalstreatedwith Methanolic leaves extract of Eucalyptus globulus plant atthe dose (200, 400 and 600mg/kg/day p.o.) and standarddrug Glibenclamide (0.5mg/kg/day i.p.) showed significantincrease in level of antioxidant enzymes like CAT, POD,SOD&GPxascomparedwithtoxiccontrolgroup.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

Table7: AntioxidantActivity

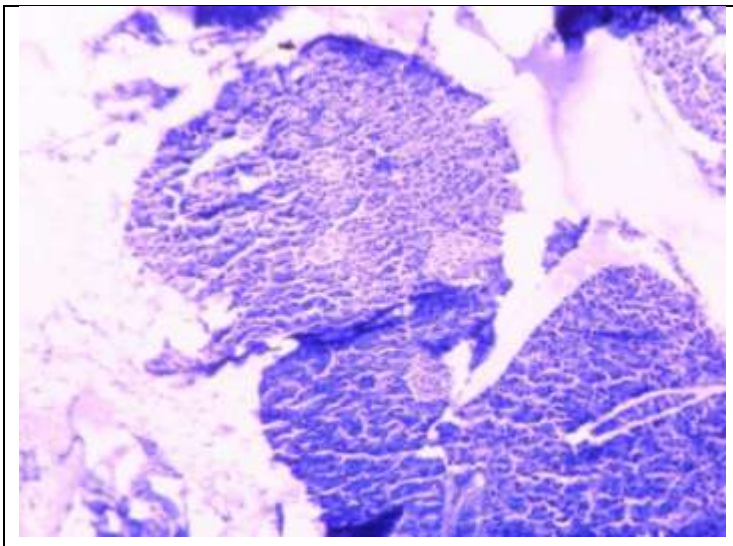
Sr.no.	Groups(n=6)	CAT(kU/mg Protein)	POD(U/mg Protein)	SOD(U/mg Protein)	GPx(U/mg Protein)
I	Control	7.9±0.73	6.5±0.50	6.8±0.76	84.4±2.0
II	Toxiccontrol	2.6±0.22	2.0±0.25	1.8±0.16	40.0±1.5
III	Standard	6.5±0.45*	5.7±0.30*	5.9±0.20*	75.6±1.4*
IV	TestgroupwithLowdoseofE.g.plantleavesextract	3.8±0.30*	3.5±0.42*	4.0±0.11*	56.0±2.5*
V	TestgroupwithIntermediatedoseofE.g.plantleavesextract	4.5±0.42*	3.9±0.25*	4.5±0.40*	61.0±2.7*
VI	TestgroupwithHighdoseofE.g.plantleavesextract	5.8±0.68*	5.5±0.33*	5.7±0.15*	72.0±2.4*

Valuesaremean±SEM, n=6,whencomparedwithnormalbyusingonewayANOVA followedbyDunnette'smultiplecomparisontest.(*p<0.05,**p<0.01).

HistopathologicalStudy:
PancreasHistopathology

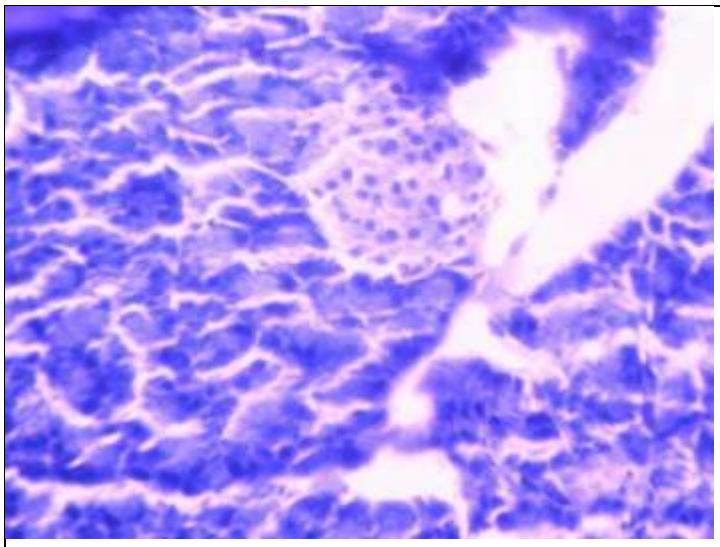
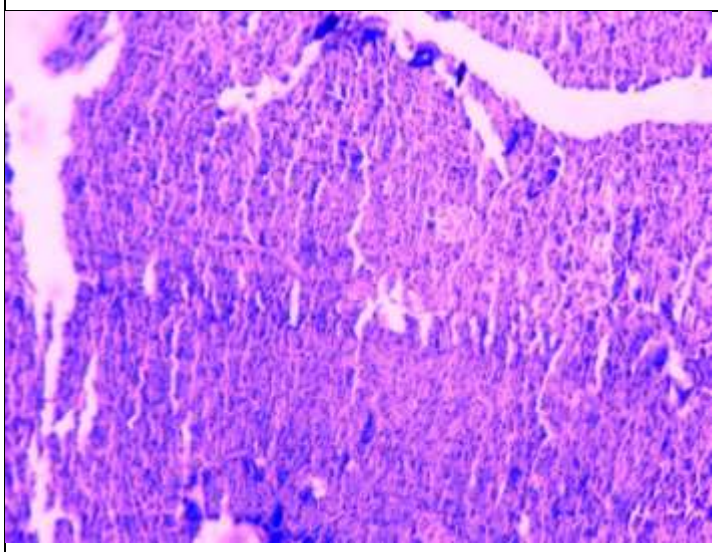
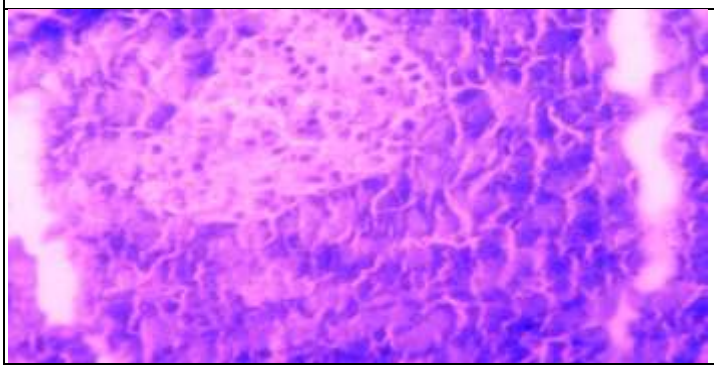
Table8: PancreasHistopathology

Sr.no.	Group	Necrosisofislets	Cellularchanges
I	Control	0	0
II	Toxiccontrol	+++	+++
III	Standard	++	+
IV	TestgroupwithLowdoseofE.g.plantleavesextract	+++	++
V	TestgroupwithIntermediatedoseofE.g.plantleavesextract	++	++
VI	TestgroupwithHighdoseofE.g.plant leavesextract	++	+

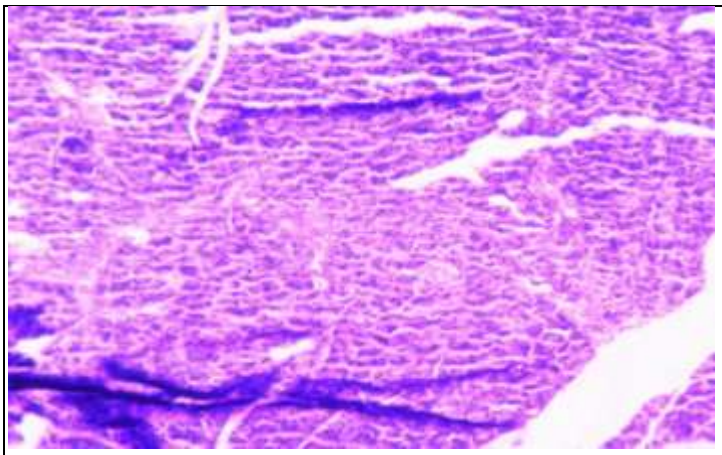
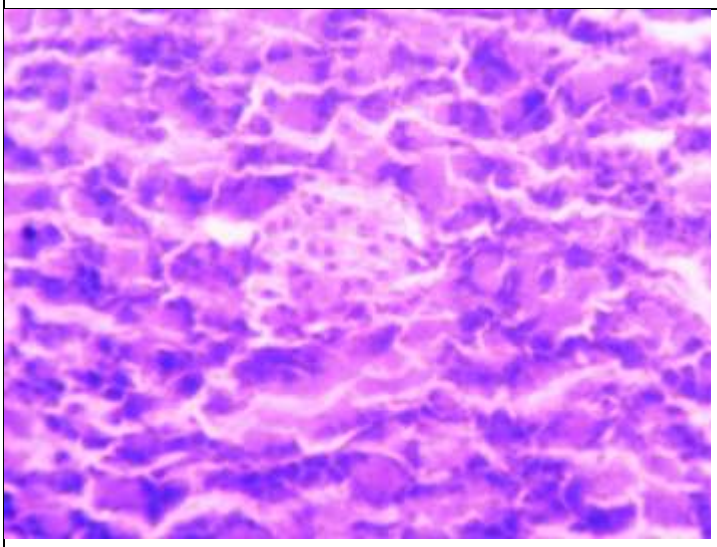
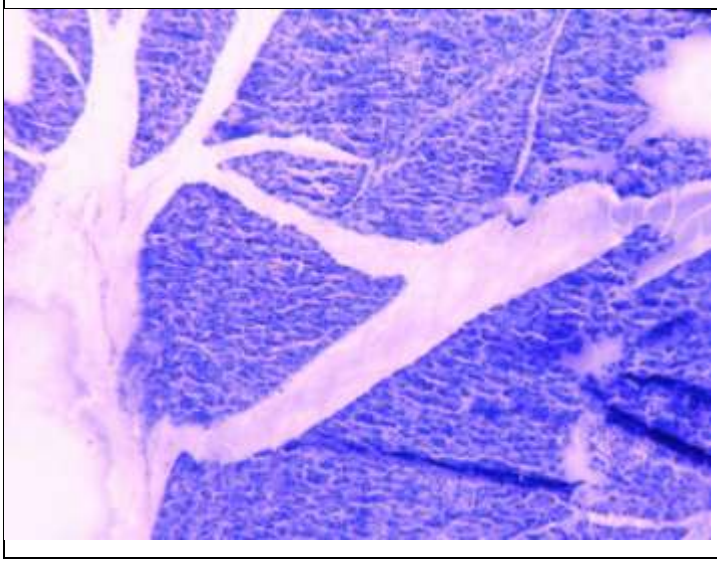


NormalcontrolPancreas-H&Estain100X

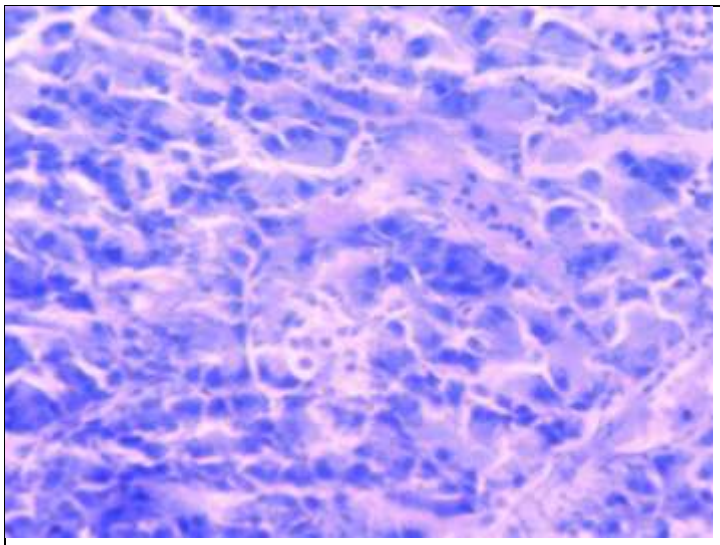
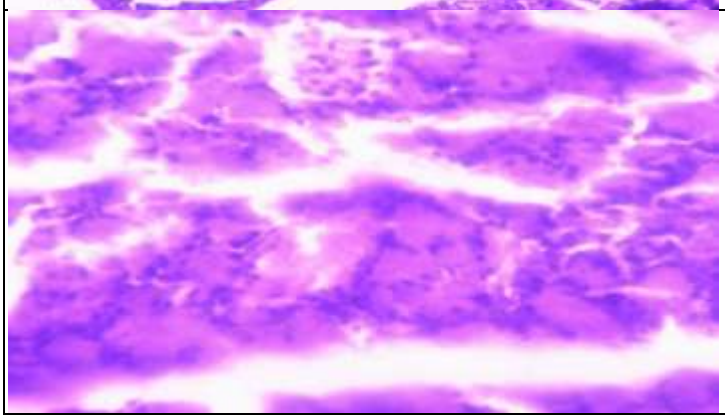
International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

	**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**

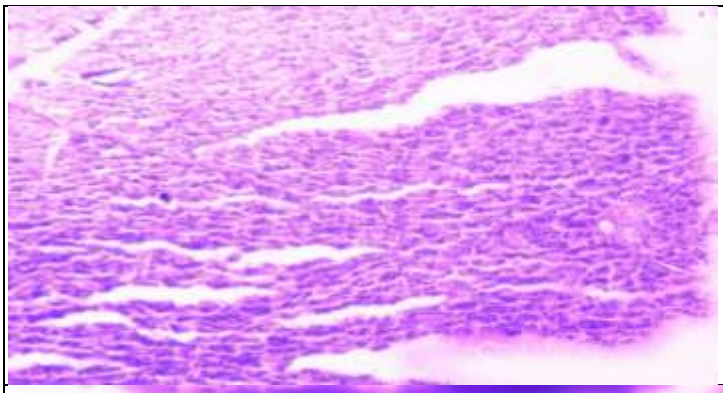
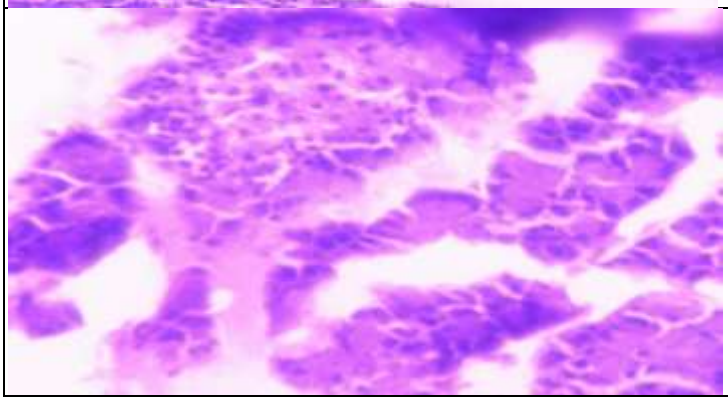
International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

	**Standard**-Photograph showing necrosis ofislets of Langerhans (black arrow), cellularinfiltration(bluearrow)H&Estain100X
	**Standard**-Photograph showing necrosis ofislets of Langerhans (black arrow), cellularinfiltration(bluearrow)H&Estain400X
	**Test – 1**-Photograph showing necrosis ofislets of Langerhans (black arrow), cellularinfiltration(bluearrow)H&Estain100X

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

	**Test – 1**-Photograph showing necrosis ofislets of Langerhans (black arrow), cellularinfiltration(bluearrow)H&Estain400X
	**Test – 2**-Photograph showing necrosis ofislets of Langerhans (black arrow), cellularinfiltration(bluearrow)H&Estain100X
	**Test – 2**-Photograph showing necrosis ofislets of Langerhans (black arrow), cellularinfiltration(bluearrow)H&Estain400X

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

	**Test – 3**-Photograph showing necrosis ofislets of Langerhans (black arrow), cellularinfiltration(bluearrow)H&Estain100X
	**Test – 3**-Photograph showing necrosis ofislets of Langerhans (black arrow), cellularinfiltration(bluearrow)H&Estain400X

IV. DISCUSSION :

AcuteToxicity,BloodGlucoseLevel&BodyWeight Determination:

Globally,therapidincreasetheincidenceofty pe2DMposes a demand for the quest of novel therapeutic drugsnecessitates addition of alternative medicine. As a resultnumber of studies has been conducted to assess theutilityof herbal medicine in type 2 DM. The present study wasundertakentoevaluatetheAntidiabeticPotentialof Methanolic Leaves Extract of Eucalyptus globules Plant inAlloxan-Induced Diabetic Albino Wistar Rats. In the LD50valuedetermination,weobservedthattheEucaly ptusglobulus plant extract was safe to use in animals. There wasno change in neurological, behavioral or autonomic, nolethality or toxic reactions were found with the selecteddoses(100, 500, 1000and 2000mg/kg/day p.o.) until theend of study period. Therefore 200, 400 & 600mg/kg wasselectedforallin vivo experimentsasmaximaldose.

The results of Hypoglycemic study have shown that theMethanolic leaves extract of Eucalyptus globulus plant atthe dose 400, 600mg/kg/day has a marked hypoglycemicpotentialascomparedwithcontrolgrou p(Table1).TheOralglucosetolerancetestinnormoglyc emicrats,blood glucose level was significantly greater in the glucoseloadedcontrolgroup.Methanolicleavesextrac tofEucalyptusglobulusplantatthedose200,400and60 0mg/kg p.o.andGlibenclamide (0.5mg/kg i.p.) reducedthe blood glucose level and improved the impaired glucosetolerance (hyperglycemia due to glucose load 2g/kg p.o.)significantly after 60 min of administration, as comparedwithcontrolgroup(Table 2).InductionofdiabetesbyAlloxanleadstolossofbody weight due to increased muscle wasting and loss of tissueproteins,whereasbodyweightofanimalssignific antlyincreasedinMethanolicleavesextractofEucalypt usglobulus plant at the dose 200, 400 and 600mg/kg/day p.oandstandarddrugGlibenclamide(0.5mg/kg/dayi.p .)treated group as compared with toxic control group (Table3).Alloxan has two distinct pathological effects: Alloxan is atoxicglucoseanalogueitselectivelyinhibitsglucose-inducedinsulinsecretionthroughspecificinhibitionof glucokinase,theglucosesensorofthebetacell,anditcau sesastateofinsulin-dependentdiabetesthroughitsability to induce ROS formation leading to demolition ofpancreasβ-cells&selectivenecrosisleadingtohypoinsulinemiaan dhyperglycemia.Theresultsoftheantidiabeticstudyha

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

veshownasignificant($p<0.05$)differencebetweentheinitialandfinalfastingbloodglucose levels of Methanolic leaves extract of Eucalyptusglobulus plant at the dose 200, 400 and 600mg/kg/day p.o.andstandarddrugGlibenclamide(0.5mg/kg/dayi.p.)treated groupsas compared with toxic control group (Table4).Thepossiblemechanismofantidiabeticactionof Methanolicextractmaybebyincreasingthepancreaticsecretionofinsulinfromtheexistingbetacells,byitsrelease from the bound form & increase in muscle glucoseuptakebyincreasedliver glucosemetabolism.

BiochemicalParametersAnalysis:

In Alloxan-induced diabetes mellitus showed improvementinbiochemicalparameters.Methanolicleavesextract of Eucalyptus globulus plant and standard drug treated groupshowedsignificantincreaseinseruminsulinlevelascompared with toxic control group (Table 5). The possiblemechanism of action of Methanolic leaves extract may beby increasing the pancreatic secretion of insulin from theexistingbetacells,byitsreleasefromtheboundform.Diabetescauseshypogonadismthroughmultiplemechanisms.Thepathogenesisoflowtestosteroneinanimalmodelswithdiabetesincludesimpairedhypothalamicsignalingandhypogonadotropichypogonadismaswellasreducedtestosteroneproductionby testicular Leydig cells. In hormonal estimation, animalstreatedwithMethanolicleavesextractofEucalyptusglobulusplant&standarddrugtreatedgroupshowedsignificant increase in testosterone level as compared withtoxiccontrolgroup(Table 5).Theserumlipidlevelsaregenerallyhighindiabetes; mappingamajorriskfactorforcoronaryheartdisease.Chronic exposure to Alloxan leads to destruction in ß cellsof pancreas and insulin resistance promotes the increase ofhormonesensitivelipaseactivity.Duetoalterationin metabolicparametersleadstoanincreaseinfatty acidsmobilizationsfromadipocytesandincreaseinhepaticsynthesisoftriglycerides,whicharereleasedintothebloodstream as VLDL, LDL cholesterol. HDL cholesterolsenrichedwith triglycerideswhich are rapidly hydrolysedandbecauseoftheirincreasedcatabolism,thebloodlevelofHDLdecreases.Inaresultoflipidprofile,markeddecrease in total cholesterol, LDL, VLDL and triglycerideswasobserved,whileincreasein HDL cholesterolwhichreduces therisk of atherosclerosis has been observedinMethanolic leaves extractEucalyptus globulusplant andstandard drug treatedgroup, which suggest that HDL isinversely related to the total body cholesterol as comparedwith toxic control group (Table 6). These results could thusreflecttheabilityofplantextractimprovethetissues ensitivity to insulin. Thus reducing the hormone sensitivelipase activity and increasing the lipoprotein lipase activity,resultinginadecreaseoflipolysistheseleading tohypolipidemicactivity.Flavonoidshavebeenshown toimprovedyslipidemia.Thusthehypolipidemiceffectof Methanolicleavesextractof Eucalyptusglobuluspl antcould be attribute to the flavonoids contained in the plant.Furthertheseextractcouldeffectivelypreventcardiovascularcomplicationsrelatedtodiabeticdyslipidemia.

Inantioxidantsstudy,Diabetesmellitussignificantlyreduced antioxidant enzymes level CAT, POD, SOD, GPxlevels. The increase in the levels of lipid peroxidation mightbe indicative of a decrease in the enzymaticantioxidantdefensemechanism.AnimalstreatedwithMethanolicleavesextractofEucalyptusglobulusplantshowedsignificantincreaseinantioxidantenzymeslevelCAT,POD,SOD&GPxas comparedwithtoxiccontrolrats(Table 7).

HistopathologicalExamination:

Inhistopathologicalstudy,thefinesectionof Alloxan-induced diabetic rats pancreas on microscopic examinationusingH&Estain,100&400Xshowedthepresenceofislets of Langerhans, blood vessels, connective tissues, interand arrangement of islets of Langerhans was normal withtightly arrangedcells anduneven distribution throughoutthelob-necrosis.Theinterlobularandintralobularductshowed widening.InstandardGlibenclamideandMethanolicleavesextractofEucalyptusglobulusplanttreatedgroupsitwasobservedthatalthoughthegapbetween the islets was more than lesser number of islets ascomparedtovehiclecontrolgroup,itwassignificantlymuchbetterthanthetoxiccontrolgroup.ThedoseofMethanolic leaves extract of Eucalyptus globulus plant atthedose(200,400and600mg/kg/day)hadimmenselyprotectedandregeneratedthecells(Table8).Thusthehistopathological examination revealed good protective andregenerativepropertyofthisherbalextract.

The results obtained with the Methanolic leaves extract ofEucalyptusglobulusplanttreatmentinchronicdiabeticmodelfurtherclarifiedtheantidiabeticpotentialofthe

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 708-721 www.ijprajournal.com ISSN: 2456-4494

extract.After14daysofMethanolicleavesextractofEucalyptus globulus plant treatment, reductions in elevatedblood glucose level, gain in body weight, hypoglycemicpotential, oral glucose tolerance, normalization in alteredbiochemicalparameters & regeneration in damagedtissueof pancreas were observed, whichcomparable with that ofthetoxiccontrolgroupaswellasstandarddrugGlibenclamide treated group. These effects could be due tothe potent bioactive Polyphenol Ellagitannins present in theplant.

V. CONCLUSION

In conclusion, it can be stated that the Methanolic leavesextractofEucalyptusglobulusplantthesourceof Ellagitannins has beneficial effects in reducing the elevatedbloodglucoselevelaswellasgainedbodyweight,hypoglycemic potential, significant oral glucose tolerance&normalizationinalteredbiochemicalparametersofAlloxan-induceddiabeticrats.Eucalyptusglobulusplantthe source of Ellagitannins associated with the stimulationof insulin secretion andenhancementofmuscleglucoseuptakeandmetabolismduetoregenerationindamagedtissue of pancreas. These effects could be due to the potentbioactivePolyphenolEllagitanninspresentintheplant.Thusjustifyingtheclaimmadebyayurvedicclassics.Therefore,Eucalyptusglobulesplantthesourceof Ellagitanninsrepresentsaneffectiveantidiabeticdietaryadjunct for the treatment of diabetes and a potential sourcefor discovery of new orally active agent for future diabetestherapy.

REFERENCES

[1]. Ramachandran A., Snehalatha C., Viswanathan V. Burden of type 2diabetes and its complications- the Indian scenario. Curr. Sci. 2002;83:1471–1476.

[2]. Jain,V.,Viswanatha,G.L.,Manohar,D.,&Shivaprasad,H.N.Isolationofantidiabeticprinciplefromfruitrindsof Punicagranatum. Evidence-Based Complementary and Alternative Medicine,2012.https://doi.org/10.1155/2012/147202

[3]. AntidiabeticActivityontheExtractsofEmbeliaribesinStreptozotocin Induced Diabetic Rats _ Insight Medical Publishing.(n.d.).

[4]. Dholi, S. K., Raparla, R., Mankala, S. K., & Nagappan, K. InvivoAntidiabetic evaluation of Neem leaf extract in alloxan induced rats.2011;01(04),100–105.

[5]. Vijayanand,S.EvaluationofAntidiabeticactivityofMurrayakoenigiionAlloxanInduced Diabeticrats.December.2016,2015.

[6]. Ito, H. Metabolites of the Ellagitannin Geraniin and Their AntioxidantActivities.2011;1110–1115.

[7]. Somani R., Kasture S., Singhai A.K. Antidiabetic potential of Buteamonosperma in rats.Fitoterapia.2006;77:86–90.

[8]. PuriD.TheinsulinotropicactivityofaNepalesemedicinal plantBiophytumsensitivum:preliminaryexperimentalstudy.JEthnopharmacol.2001;78(1):89–93.

[9]. Pari L., Maheswari J.U. Hypoglycemic effect of Musa sapientum L. inalloxan-induceddiabeticrats.J.Ethnopharmacol.1999;68:321–325.

[10]. Chaudhary, S., Khosa, R. L., Jha, K. K., & Verma, N. Evaluation ofAntidiabeticActivityofCressa Cretica.2010;188,181–188.

[11]. King,A.J.F.Theuseofanimalmodelsindiabetesresearch.https://doi.org/10.1111/j.2012;1476-5381,01911.x

[12]. Glugliano D., Ceriello A., Paolisso G. Oxidative stress and diabeticvascularcomplications. Diabet.Care.1996;19:257–267.

[13]. Rats, D.Evaluation of Antidiabetic Activity of Ethanolic Extract ofAjuga Parviflora inDiabeticRats.2019; 9(4),112–115.

[14]. Mathew P.T., Augusti K.T. Hypoglycemic effects of onion, Alliumcepa Linn. on diabetes mellitus- a preliminary report. Ind. J. Physiol.Pharmacol. 1975; 19:213–217.