

ISSN **0976-9595** Research Article

Available online through http://www.sciensage.info

PHYTOPHARMACOLOGICAL EVALUATION OF *DALBERGIA LATIFOLIA* ROXB FOR ANTIDIABETIC ACTIVITY AND ITS EFFECT ON LIPID PROFILE AND HEPATIC ENZYMES OF GLUCOSE METABOLISM IN DIABETIC RATS

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ABSTRACT

It is essential to gauge the herbal plants scientifically and proper documentation should be made to understand their medicinal properties. In current scenario there exists a very high demand for the herbal products in the entire world that led to the extensive research in many pharmaceutical industries on plant materials for the exploration of potential medicinal value. This inclination seems to be a result of people finding various alternative systems of drugs. A large number of drugs are reported to have excellent medicinal value and are in use for the treatment of diabetes. *Dalbergia latifolia* Roxb is evergreen, deciduous tree belongs to fabaceae family, it consist of white small flower and identified by grey bark which peels in long fibers. In the present study, qualitative and quantitative phytochemical screening of Hydroalcoholic extract of bark of *Dalbergia latifolia* (DLExt) and pharmacological studies involved acute oral toxicity study, oral glucose tolerance test (OGTT) hypoglycemic activity and antidiabetic activity of DLExt was done. The results suggested that *Dalbergia latifolia* (DLExt) induced a potent reduction in glycaemia exhibited potential to reduce the blood glucose level (BGL). The study showed that Hydroalcoholic extract of bark of *Dalbergia and* in pancreas and in kidney and enhances SOD, CAT, GPx and GST activity. The involvement of DLExt reduction in level of TG, TC, LDL, VLDL and elevation in HDL level in diabetic rats was also demonstrated. The study was supported with histopathology studies and showed remarkable improvement in pancreatic tissues, normal architecture of hepatocytes and normal architecture of bowman's capsule and renal tubules.

Keywords: Dalbergia latifolia Roxb, Phytochemical screening, Hypoglycemic activity, Antidiabetic activity.

1. INTRODUCTION

India features a century's old tradition of using medicinal plants and herbal medicines for the alleviation of varied diseases and ailments. Considering the number of plant species that it is found that there are around 45,000 plant species and out of these species, many are claimed to possess medicinal properties. People often look towards the normal systems of drugs not just for the curative effects of plants, but also to hopefully provide them healthiness. Plants are considered as an exemplary source of drugs. Ayurveda and other Indian literature mention the utilization of plants in treatment of varied human ailments [1].

Diabetes is a well known disorder, it usually occurs after the age of 35-45 among obese people. Diabetes mellitus can be defined as a collective group of syndrome commonly characterized by hyperglycemia along with altered metabolism of lipids, carbohydrates as well as proteins. Diabetes causes an increased risk of complications and commonly effecting eyes, kidneys, nerves and arteries. Due to increase of sugar in blood and urine diabetic condition is created. In normal condition sugar level in blood is 80-120 mg/100 cc of blood on fasting and up to 160mg/100 cc of blood in the postprandial state [2].

As per Ayurveda and Hindu literatures like *Charka* Samhita, Madhav Nidan and Astang Sanghra, there are about 600 plants, which are supposed to have antidiabetic property. From many years herbal medicines are used in almost all cultures. Ayurvedic anti-diabetic herbs are responsible for improving digestive power through the increase of *Rasas* (gastric secretions), *laghu* and *ruksha*. It also reduces output of overall body fluids like urine, sweat.

Dalbergia Latifolia Roxb is an indigene to southeast India. It is identified by its common names like Black Rosewood, Indian Rosewood and Sheesham. Active ingredients obtained from *Dalbergia Latifolia* Roxb uses in much disease treatment traditionally over long period of time and recent studies also shows its potential effect in treatment of various chronic diseases like leprosy, diabetes and cancer [3].

The present study was focused on the evaluation of *Dalbergia latifolia roxb* an important Indian medicinal plant for antidiabetic activity and scientific exploration of ethnopharmacological claims. The determination of the effect of hydroalcoholic extract of bark of *Dalbergia latifolia* roxb on lipid profile and hepatic enzymes of glucose metabolism in diabetic rats model was carried out in order to support the antidiabetic potential of the plant.

Taxonomical Hierachy

Domain - Eukaroyta Kingdom - Plantae Phylum - Spermatophyta Sub phylum - Angiospermae Class - Dicotyledonae Order - Fabales Family - Fabaceae Sub family - Dalbergia Genus - Dalbergia

2. MATERIAL AND METHODS

2.1. Plant material

The selection of plant species for this study was based on their traditional use for diabetes treatment, the information being culled from published sources and traditional healers. The selected plant bark portions were selected for the present studies (Table 1).

Table 1: Plant material

| Name of plant | me of plant Common name | | Parts used |
|-----------------------------|-------------------------|----------|---------------|
| Dalbergia latifolia Roxb | Indian rosewood | Fabaceae | Bark |

2.2. Collection, identification and authentification of plant parts

The plant part (bark) of *Dalbergia latifolia Roxb* as collected from forest area of Indore region, Madhya Pradesh, India and authentified by Dr. S.N. Dwivedi, Head of the Department, Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P. India and voucher specimen number J/Bot./2019-005PGB (*Dalbergia latifolia* Roxb, bark part) was preserved in institute department for future reference.

2.3. Preparation of extracts

The extraction yield of the extracts from plants species highly depends on the solvent polarity, which determines both qualitatively and quantitatively the extracted compounds. Ethanol, and water are the most widely used solvent for the extraction because both the solvents are found to possess low toxicity along with high extraction yield. Even these solvents have advantage of modulating the polarity by using mixtures in different ratios.

Authenticated bark of plants were dried in hot air oven, chopped, pulverized and subjected for extraction, separately. Dried powder of various plant parts were extracted with mixture of 50% ethanol+50% distilled water in a soxhlet extractor. The extraction process was continued till the solvent available in the thimble became clear. Once the process of extraction was complete, the extract was filtered and the solvent was distilled off by the help of rotary evaporator at the temperature of around 50°C. The extract was freeze dried and percentage yield was calculated. The percentage yields of the extracts were calculated with reference to air dried powder. The extracts were allowed to be preserved in a refrigerator and used for future studies. Some part of the total extract was used for qualitative and quantitative phytochemical investigation and rest of the extract was used for preliminary pharmacological screening. The extract code/nomenclature used for further studies are DLExt (Hydroalcoholic extract of bark of Dalbergia latifolia).

2.4. Qualitative and quantitative phytochemical screening of DLExt

2.4.1. Qualitative phytochemical screening of various extracts of DLExt

DLExt (Hydroalcoholic extract of bark of *Dalbergia latifolia*) was subjected to qualitative chemical analysis to detect the presence of various phytoconstituents [4]. The various tests and reagents used are given below (Table 2).

2.4.2. Quantitative phytochemical screening of DLExt

2.4.2.1. Estimation of total phenolics content

Phenols react with phosphomolybdic acid in Folin-Ciocalteau's reagent in alkaline medium that results in the formation of blue-colored complex (molybdenum blue). The absorbance measurement can be done spectrophotometrically at 760 nm.

Total phenolics in **DLExt** was determined with Folin-Ciocalteu's reagent. One ml of extract (1mg/ml in distilled water) was taken in separate volumetric flask and one ml of Folin-Ciocalteu reagent was added in flask. After three minute, three ml of 2% Na₂CO₃ was added. Subsequently, the mixture was shaken for two hours at room temperature and absorbance was read at 760nm

and experiment was performed in triplicate [5]. A = 0.001C+0.0033Where, A is absorbance and C is concentration, (GAE) g/100 gm of dry matter.

| Phytoconstituents | Test |
|--|---|
| Tests for carbohydrates | Molish's test, Fehling's test, Benedict's test |
| Tests for proteins and amino acids | Biuret test, Million's test, Xanthoproteic test |
| Tests for glycosides | Legal's test, Keller-Killiani test (test for deoxy sugars), |
| Test for anthraquinone glycosides | Borntrager's test |
| Test for flavonoids | Shinoda test, Alkaline reagent test |
| Test for saponins | Foam test |
| Test for alkaloids | Mayer's test, Hager's test, Dragendorff's test, Wagner's test |
| Test for steroids and triterpenoids | Libermann-Burchard's test, Salkowski's test |
| Tests for tannins (phenolic compounds) | Ferric chloride test, Gelatin test, Lead acetate test |

Table 2: Qualitative phytochemical screening tests

2.4.2.2. Estimation of total flavonoids content

Total flavonoids content in **DLExt** was measured by aluminum chloride colorimeter assay. In this method the flavonoids react with aluminum chloride reagent in order to produce a colored product that be measured by the help of spectrophotometer at 510nm.

One gm of extract, was macerated with 100 ml methanol (hot decoction) for one hr followed by filtration. One ml of each extract was placed in 10 ml volumetric flask and three ml of methanol and 0.3 ml $NaNO_2$ was added and after five minutes, three ml of $AlCl_3$ was added. Two ml of 1M NaOH was added after six min and total volume was made upto 10 ml with methanol. The absorbance was quantified against a blank at 510nm and the total flavonoids content was calculated using following equation [6].

A = 0.01069C - 0.001163

Where, A = absorbance, C = flavonoids content (CE) g/100 gm of dry matter

2.5. Pharmacological studies of DLExt

Pharmacological studies involved acute oral toxicity study, oral glucose tolerance test (OGTT) hypoglycemic activity and antidiabetic activity of DLExt.

2.5.1. Experimental animals

Healthy adult wistar albino rats (150-200 gm) were used for the study. The animals were stabilized for 1 week, housed in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, $25\pm30^{\circ}$ C), the animals were fed with standard pellet diet and water *ad-libitum* throughout the course of the study. The handling of the animals were done gently in order to avoid the stress condition and causes increased adrenal output. The experiment was approved by Institutional Animal Ethical Committee of B.R. Nahata College of Pharmacy, Mandsaur with letter number IAEC/BRNCOP/2020/011 with CPCSEA registration number 918/PO/Re/S/05/CPCSEA. The normoglycemic animals were selected for this experiment having the fasting blood glucose level around 80 mg/dL. The hyperglycemic animals were selected having fasting blood glucose concentration around 200-300 mg/dL.

2.5.2. Preparation of the dose

The suspension of DLExt was freshly prepared in tween 80 and suspended in distilled water and glibenclamide 5 mg/kg p.o was prepared in tween 80, suspended in distilled water.

2.5.3. Acute oral toxicity study (OECD Guidelines, 2001) [7]

The acute toxicity test aims at establishing the therapeutic index. The acute toxicity study was conducted as per OECD guidelines 420- Fixed Dose Procedure (FDP), as in annex 2D. The prepared suspension of DLExt was administrated orally to overnight fasted swiss albino mice (n=6) at dose of 2000 mg/kg b.w. respectively. The observation of animals was done continuously for the initial 4hrs for behavioral changes and mortality. The intermittent observation for the next 6 h was done and then again at 24 h and 48 h after dosing for 14 days. The common behavior parameters that were observed, included convulsion, hyperactivity, sedation, grooming, loss of

righting reflex and increased respiration.

Selection of Doses: In this study dose of 2000 mg kg⁻¹ was found to be safe for hydroalcoholic extract of bark of *Dalbergia latifolia*, therefore extracts at dose of 250 mg kg⁻¹ and 500 mg kg⁻¹ were chosen for the further experimentation.

2.5.4. Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) measurement is responsible for assessing the ability of body to use glucose as main energy source. The test is performed to simplify and also to facilitate the diagnosis of diabetes. Oral glucose tolerance test is commonly referred as physiological induction of diabetes mellitus, since the level of blood glucose of the animal is transiently enhancing without damage to the pancreas.

Overnight fasting normal rats were allowed to be divided into four groups in such a way that in each group six animals were available (n=6). Group I served as a control; received vehicle (Tween 80 in distill water). Group II served as standard group; received Glibenclamide (5 mg kg⁻¹p.o.) suspended in vehicle and Group III received 250 mg kg⁻¹ oral dose of Hydroalcoholic extract of bark of *Dalbergia latifolia*, Group IV received 500 mg kg⁻¹ oral dose of Hydroalcoholic extract of bark of *Dalbergia latifolia* (Table 3).

All animals were loaded with glucose $(1.5 \text{ g kg}^{-1}, \text{ p.o.})$ 30 min after the extract and drug administration. The blood samples were collected by snipping tail with surgically sterilized needle. Blood glucose was determined just prior to glucose administration (0h) and 1, 2, 3 and 6h after glucose administration. Blood glucose concentration was estimated by the glucose oxidase enzymatic method, using Accu-chek Active TM Test strips in Accu-chek Active TM Test meter.

2.5.5. Hypoglycemic activity

The hypoglycemic activity is significant in the diagnosis of diabetes mellitus. It measures the ability of drug to reduce blood glucose level. This method allows for the effect of the drug to be tested in the animal with an intact pancreatic activity. The comparison may give some information regarding mechanism of action. Experimental animals were divided into four groups of six animals per group (n = 6). Group I served as a control; received vehicle (Tween 80 in distill water). Group II served as standard group; received Glibenclamide (5 mg kg⁻¹p.o.) suspended in vehicle and Group III received 250 mg kg⁻¹ oral dose of Hydroalcoholic extract of bark of Dalbergia latifolia, Group IV received 500 mg kg⁻¹ oral dose of Hydroalcoholic extract of bark of Dalbergia latifolia, suspended in vehicle (Table 4).

The blood glucose level of all experimental rats was determined at 0 hr (before oral administration), by snipping tail with surgically sterilized needle and then after 1, 2, 3, 4 and upto 6 h respectively.

| Group no. (n=6) | Group | Treatment | | | |
|-----------------|-------------------|---|--|--|--|
| Group-I | Control | Vehicle Treated (Tween 80 in distill water) | | | |
| | | Standard group and received Glibenclamide (5 mg kg-1p.o.) suspended | | | |
| Group-II | rositive Collitor | in vehicle | | | |
| Crown III | Extract Treated | Treated with Hydroalcoholic extract of bark of Dalbergia latifolia, | | | |
| Group-III | | DLExt 250mg/kg | | | |
| Crown IV | Eutro at Tuested | Treated with Hydroalcoholic extract of bark of Dalbergia latifolia, | | | |
| Group-Iv | Extract Treated | DLExt 500mg/kg | | | |

Table 3: Effect of DLExt on glucose loaded hyperglycemic rats

Table 4: Effect of DLExt on normoglycaemic rats

| Group no. (n=6) | Group | Treatment | | | |
|-----------------|-------------------|---|--|--|--|
| Group-I | Control | Vehicle Treated (Tween 80 in distill water) | | | |
| | | Standard group and received Glibenclamide (5 mg kg-1p.o.) suspended | | | |
| Group-II | rositive Collitor | in vehicle | | | |
| Crown III | Extract Treated | Treated with Hydroalcoholic extract of bark of Dalbergia latifolia, | | | |
| Group-III | Extract Treated | DLExt 250mg/kg | | | |
| Crown IV | Extract Treated | Treated with Hydroalcoholic extract of bark of Dalbergia latifolia, | | | |
| Group-IV | Extract Treated | DLExt 500mg/kg | | | |

2.5.6. Antidiabetic activity

2.5.6.1. Induction of diabetes

Overnight fasted rats were made diabetic by single intra peritoneal injection of freshly prepared solution of alloxan monohydrate in normal saline at a dose of 120 mg/kg b.w. Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, in order to stave off the hypoglycemia during the first day of alloxan administration rats were treated with 20% glucose solution (5-10 mL) orally after 6 hours for the next 24 hours. After 5 days blood samples was collected and blood glucose levels were determined to confirm diabetes. The rats with blood glucose level > 250 mg/dL were considered to be diabetic and were used in experiment.

Diabetic rats were divided into four groups of six animals each (n=6). Group I served as a control; received vehicle (Tween 80 in distill water). Group II, diabetic control; received vehicle (Tween 80 in distill water). Group III received glibenclamide as reference drug (5 mg/kg p.o.) suspended in vehicle. Group IV diabetic rats received 500 mg kg⁻¹ oral dose of Hydroalcoholic extract of bark of *Dalbergia latifolia* suspended in vehicle. All treatments were continued for 21 days (Table 5).

| Table 5, Effect of DEEXt of DOL of anoxali induced diabetic rats | Table 5: | Effect of | f DLExt or | n BGL of | alloxan | induced | diabetic rats |
|--|----------|-----------|------------|----------|---------|---------|---------------|
|--|----------|-----------|------------|----------|---------|---------|---------------|

| GROUPS (n=6) | GROUPS | TREATMENT |
|--------------|-----------------------|---|
| Group-I | Control | Normal rats given vehicle only |
| Group-II | Diabetic control | Diabetic rats given vehicle only (Diabetic control) |
| Group-III | Standard Drug Treated | Diabetic rats given Glibenclamide (5 mg kg ⁻¹ p.o.) (Standard) |
| Group-IV | Extract Treated | Treated with Hydroalcoholic extract of bark of <i>Dalbergia latifolia</i> , DLExt 500mg/kg |

2.5.6.2. Estimation of blood glucose levels

Extracts were given orally to the animals once before food was given. The blood glucose concentrations of the animals were determined at the beginning of the study and the measurements were repeated on at 0th, 5th, 15th and 21st day after the initial of the experiment. Blood glucose concentration was estimated by the glucose oxidase enzymatic method, using Accu-chek Active TM Test strips in Accu-chek Active TM Test meter.

2.5.6.3. Evaluation of changes in body weight

The body weights of experimental animals were recorded on 0^{th} and 21^{st} day of the experiment.

2.5.6.4. Collection of serum and tissue samples

At the end of experimental period, rats were deprived of food overnight and sacrificed by diethyl ether anesthesia. Blood was collected by heart puncture. The blood samples of each animal were taken and allowed to clot for 45 minutes at room temperature and serum was separated by centrifugation at 4500rpm for 10 min. The serum samples were collected and left standing on ice and analyzed for biochemical parameters including insulin and lipids. The tissues (liver, pancreas and kidney) were excised and transferred into icecold containers for biochemical estimations and histopathology. 2.5.6.5. Estimation of serum insulin levels

The serum insulin levels were determined using rat insulin ELISA kit [14] (Mishra et al., 2010). The ADVIA Centaur (IRI) and Ready Pack, of Bayer of corporation diagnostic kit were used for the estimation of insulin, which follows immunoassay using two monoclonal antibodies.

2.5.6.6. Estimation of lipids in serum

Serum was analyzed for serum total cholesterol (TC), total glycerides (TG), high density lipoprotein (HDL) were estimated using standard enzymatic colorimetric kits (Span diagnostics Ltd., India) [15] (Bagri et al., 2009), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated using formula.

2.5.6.7. Estimation of serum total cholesterol

The estimation was done by the help of Span diagnostic kit that utilizes cholesterol oxidase/peroxidase (CHOD-POD) method.

In this method the enzyme i.e cholesterol esterase is responsible for catalyzing the hydrolysis of cholesterol esters in order to free cholesterol and fatty acid molecules. Later the free cholesterol oxidizes in the presence of cholesterol in order to form cholesten-3one and H_2O_2 . Liberated H_2O_2 reacts with phenol and 4AAP in presence of peroxidase to form red colored quinoneinine complex the color intensity was measured at 505 nm [8, 9].

2.5.6.8. Estimation of serum of triglycerides

Span diagnostic kit was used for estimation of triglycerides, which followed end point colorimetric enzymatic test using glycerol-3-phosphate oxidase.

2.5.6.9. Estimation of serum high-density lipoprotein cholesterol (HDL-C)

Span diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase/ peroxidase (CHOD-POD) method.

2.5.6.10. Estimation of serum low-density lipoprotein cholesterol (LDL-C)

Using the data obtained including total cholesterol, HDL cholesterol and VLDL, the LDL cholesterol levels were calculated using the empirical equation of Friede Wald.

Calculation:

Serum LDL cholesterol = Total cholesterol - (HDL cholesterol + VLDL cholesterol)

2.5.6.11. Estimation of serum very low-density lipoprotein cholesterol (VLDL-C)

Using the data obtained including triglycerides, the VLDL cholesterol levels were calculated using empirical equation of Friede Wald.

Calculation:

Serum VLDL cholesterol = Triglycerides/5

2.5.6.12. Determination of the lipid peroxidation

The pancreas and kidney were homogenized in 0.025 M Tris-HCl buffer, pH 7.5 after centrifugation at 10,000 X g for 10 min, the clear supernatent was allowed to be used for the estimation of thiobarbituric acid reactive substances (TBARS).

Malondialdehyde was formed due to the breakdown of polyunsaturated fatty acids (PUFA) and serves as a convenient index in order to determine the extent of peroxidation reaction. Malondialdehyde reacts with thiobarbituric acid (TBA) in order to form thiobarbituric reacting substance (TBARS) which is pink chromogen, quantified at 532 nm.

Acetic acid; 1.5ml (20%, pH 3.5), 1.5 ml of thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulphate (8.1%) was added to 0.1ml of supernatant and heated at 100°C for 60min. Mixture was cooled and five ml of n-butanol pyridine (15:1) mixture was added with

one ml of distilled water and vortexed vigorously. After centrifugation at 1200 x g for 10 minute, separation of organic layer was done and absorbance was allowed to be measured at 532 nm. It was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as nanomoles of TBARS, mg of protein.

Calculations were made as per the formula: Inhibitory rate = $[1 - (A_1 - A_2)/A_0] \times 100$

Where A_0 was the absorbance of control {without extract} and A_1 was the absorbance in the present of the extract, A_2 was the absorbance without liver homogenate (Wang et al., 2008).

2.5.6.13. Estimation of in vivo antioxidant activity

Liver was rinsed in ice-cold saline to remove the blood and immediately freezed and stored at -80° C in deep freezer. The liver was homogenized in 50mM phosphate buffer solution (pH 7.4) using a tissue homogenizer at 40°C. The homogenates were centrifuged at 15,000×g for 20 min and supernatant was used for analyses of glycogen, enzymatic and nonenzymatic antioxidants.

2.5.6.13.1. Estimation of enzymatic antioxidants

2.5.6.13.1.1. Estimation of Superoxide Dismutase (SOD)

In this method the assay mixture was having 0.1mL of supernatant, 1.2 mL sodium pyrophosphate buffer of pH 8.3, along with 0.1mL of phenazine methosulphate (186 μ m), 0.3mL of nitro blue tetrazolium and 0.2mL of 750 μ M NADH. The initiation of reaction was done through the addition of NADH. Post incubation at 30°C for time period of around 90 seconds, the reaction was allowed to be stopped through the addition of 0.1mL of glacial acetic acid. Later, the reaction mixture was allowed to stir vigorously with 4.0mL of n-butanol solution. The intensity of color of the chromogen in the butanol was allowed to be measured by the help of spectrophotometer at 560 nm. The concentration of Superoxide dismutase was expressed as U/mg of protein.

SOD (U/3ml) = { $(A-B) \times 100/(A \times 50)$ }

Where, A=Control group, B= Treated group, Unit \times 10 = Unit/ml sample solution

2.5.6.13.1.2. Estimation of Catalase

A 0.1 ml supernatant was added to cuvette that contains around 1.9 ml of phosphate buffer (50 mM) of pH 7.4. The initiation of reaction was done through the addition of 1 ml of freshly prepared hydrogen peroxide (30 mM). The rate of decomposition of H_2O_2 was measured at spectrophotometrically 240 nm. Activity of catalase was expressed as nanomole/min/litre. *Calculation:*

Catalase (U/L) = Absorbance \times F Factor (F)=Total volume (ml)/Sample volume (ml)/43.6 F = 0.688

2.5.6.13.1.3. Estimation of Glutathione peroxidase (GPx) activity

The reaction mixture consisted of 400μ L, 0.25M potassium phosphate buffer (pH 7.0), 200 μ L supernatant, 100 μ L GSH (10mM), 100 μ L NADPH (2.5mM) and 100 μ L Glutathione reductase (6U/ml). The reaction was started by adding 100 μ L Hydrogen peroxide (12mM) and absorbance was measured at 366nm at 1 min interval for 5 min. using a molar extinction coefficient of 6.22 x10³ M⁻¹ cm⁻¹. Data was expressed as mU/mg of Protein.

2.5.6.13.1.4. Estimation of Glutathione-s-Transferase (GST)

Glutathione-S-transferase (GSTs) is an important cellular antioxidant which indirectly inhibit the free radicals [10]. The reaction mixture consisted of 2.75 ml of sodium phosphate buffer (pH 7.4), 0.1 ml reduced glutathione (1mM), 0.1 ml supernatant in a total volume of 3.0 ml. The change in absorbance were measured at 340 nm and the enzyme activity was expressed as nanomoles of 1- Chloro 2,4-dinitro benzene (CDNB) conjugated formed/min/mg protein using a molar extinction coefficient of 9.6 x10³ M^{-1} cm⁻¹.

Calculation:

GST (U/L) = (Absorbance/0.0096) × 30 × 10 Where, Molar absorption coefficient = 0.0096, Dilution factor = 10

- 2.5.6.13.2. Estimation of non enzymatic antioxidants activity
- 2.5.6.13.2.1. Glutathione Synthetase (GSH or Reduced Glutathione)

Reduced glutathione is an important cellular antioxidant. It maintains the normal structure of red blood cells and keeps hemoglobin in the ferrous state. It is involved in detoxication process. The toxic substance (organophosphate, nitro compound) are converted into mercapturic acid. Glutathione is involved in maintaining normal brain function [11].

To 0.01ml of supernatant, two ml of phosphate buffer (pH 8.4), 0.5ml of 5,5 di thio bis -2 nitro benzoic acid

(DTNB) and 0.4ml of double distilled water was added. Mixture was vortexed and absorbance was read at 412 nm with in 15 min. The concentration of reduced glutathione was expressed as μ g/mg of protein

Calculation

GSH (Mole/litre) = (Absorbance/14700) x 50 Where, Molar absorption coefficient = 14700, Dilution factor = 50

2.5.6.13.2.2. Estimation of Glycogen in liver

The tissue samples were allowed to be rinsed twice with the help of ice cold saline and further solubilized by incubating it with 30% KOH at temperature of around 55°C for time period of 30 min. After neutralization with 1N HCl, 0.2 ml of cell suspension along with 0.8 ml H₂O, and two ml anthrone reagent (0.2 g anthrone/100 ml 95% H₂SO₄) were mixed on ice. The incubation was done at temperature of 100°C for time period of 10 min, and placed on ice. Absorbance was measured at 620 nm [12] and expressed as milligrams of glycogen per gram tissue.

2.5.6.14. Statistical Analysis

The data were expressed as mean \pm SEM. The data of antidiabetic activity were analyzed by one way analysis of variance (ANOVA) followed by "Dunnet's test." p value less than 0.05 was considered as statistically significant.

2.5.6.15. Histopathological studies of pancreas, liver and kidney

Histopathological studies of the pancreas, liver and kidney were conducted in control and diabetic control, and extract treated groups that showed significant pharmacological activity for 21 day.

On day 22nd when the animals were sacrificed, the pancreas, liver and kidney of all animals from each group was excised and washed with saline solution, soaked in filter paper and transferred into 10% formalin solution for histopathological studies [13].

3. RESULTS AND DISUSSION

3.1. Percentage yields and appearance of DLExt

The percentage yields of different extracts were calculated with reference to air dried powder and reported in % w/w. Percentage weight by weight indicates the number of parts by weight of active ingredient contained in total weight of solution or mixture considered as 100 parts by weight (Table 6).

| Extracts | Percentage yield % w/w | Appearance |
|--|---------------------------|------------|
| Hydroalcoholic extract of bark of <i>Dalbergia</i> | 11.76% | Brown |
| latifolia (DLExt) | | |

Table 6: Percentage yields and appearance ofextract

3.2. Qualitative and quantitative phytochemical screening of DLExt

3.2.1. Results of qualitative phytochemical screening of DLExt

The preliminary phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using precipitation and coloration reaction to identify the major natural chemical groups. General reactions in this analysis revealed the presence or absence of these compounds in the crude extracts tested. Summary of preliminary phytochemical screening of extract is given in table 7.

Table 7: Results of qualitative phytochemicalscreening of various extracts of DLExt

| Extracts | Phytochemicals |
|------------------------------------|--------------------------|
| Hydroalcoholic extract of | Carbohydrates, proteins, |
| bark of <i>Dalbergia latifolia</i> | amino acids, phenolic |
| (DLExt) | compounds and flavanoids |

3.2.2. Results of quantitative phytochemical screening of DLExt

Plant phenolics are the major group of compounds acting as primary antioxidants and help to treat or manage metabolic disorders like diabetes.

Table 8: Results of quantitative phytochemicalscreening of extract

| 0 | | |
|-------------------|---------------|---------------|
| | Total | Total |
| | Phenolic | flavonoid |
| Extracts | content (TP) | content (TF) |
| | (GE) g/100 gm | (CE) g/100 gm |
| | of dry matter | of dry matter |
| Hydroalcoholic | | - |
| extract of bark | 7.04 | ()5 |
| of Dalbergia | 7.04 | 0.35 |
| latifolia (DLExt) | | |

Flavonoids, one of the most diverse and widespread group of natural compounds, are probably the most important natural phenols. These compounds encompass a broad spectrum of chemical and biological properties like management metabolic disorders like diabetes. The results of quantitative determination of phytoconstituents of DLExt is given in table 8.

3.3. Pharmacological studies of DLExt

3.3.1. Results of acute oral toxicity studies of DLExt Results of acute toxicity studies on albino mice showed no mortality at a dose of 2000 mg/kg of hydroalcoholic extract of bark of *Dalbergia latifolia*, during observation time period of 14 days. Based on these results dose of 2000 mg kg⁻¹ was found to be safe for therefore extracts at dose of 250 mg kg⁻¹ and 500 mg kg⁻¹ were chosen for the further experimentation.

3.3.2. Effect of various extracts of DLExt on BGL of glucose loaded hyperglycemic rats (oral glucose tolerance test, OGTT)

The blood glucose level (BGL) DLExt, glibenclamide and vehicle treated albino rats after oral administration of glucose (1.5 mg kg⁻¹) are summarized in table 9. The blood glucose level of normoglycaemic rats acquired peak after 1 hr of oral administration of glucose and gradually decreases to the preglucose load level. Glibenclamide prevented the severe increase in glucose level 1 hr after glucose loading and reduced blood glucose level of animals even below the normal values in 3^{rd} and 6^{th} hr. DLExt (500mg/kg) induced a potent reduction in glycaemia with maximum fall may be due to increase in insulin concentration in glucose loaded rats. On the other hand glibenclamide showed more marked insulin tropic effect.

3.3.3. Effect of DLExt of normoglycaemic rats (hypoglycemic activity)

The blood glucose levels were compared to the values obtained from control group. Oral administration of vehicle did not change the BGL. Glibenclamide (5 mg kg⁻¹) induced significant reduction in blood glucose level when compared to control group. DLExt transiently decreases basal glucose respectively after 6 hr of oral administration may be due to insulin increasing effect.

Observed results of both the activities, clearly indicates that DLExt (500mg/kg) exhibited maximum potential to reduce the BGL in both models. The plants exhibit different mechanism of action to reduce blood glucose level. Some exhibits properties similar to sulfonylurea's drugs to reduce BGL in normoglycaemic animals by increasing the secretion of insulin from pancreas, while others act like biguanides which don't affect blood glucose in normal states. The study depict that DLExt extracts could have sulphonylurea like mechanism. Results are summarized in table 10.

3.3.4. Induction of diabetes and Effect of of DLExt of on BGL of alloxan induced diabetic rats

Alloxan, a beta cytotoxin induces chemical diabetes by damaging the insulin secreting cells of the pancreas. Alloxan (120 mg/kg) was selected in order to destroy insulin secreting β cells in the islets of langerhans and their effect is irreversible. The sudden rise in blood insulin concentration after alloxan injection and it is of

short duration and is followed by complete suppression of islet response. Results of study showed that in G-I glucose level did not change, whereas in G-II BGL rises due to prolonged production of hyperglycaemia. G-IV exhibited more significant antidiabetic effect as evident by reduction of BGL. The possible mechanism by which the plant extract mediates antidiabetic action might be potentiation of pancreatic secretion of insulin from existing residual β cell of islets or due to enhanced transport of blood glucose to peripheral.

Results of antidiabetic activity is summarized in table 11.

| Creanna | Blood Glucose level (mg/dl) | | | | | | |
|--|-----------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|--|
| Groups | 0hrs. | 1 hrs | 2 hrs | 3 hrs | 4hrs | 6 hrs | |
| G-I -ve control (Glucose 1.5g/kg) | 75.22±1.67 | 141.57±1.43 | 121.46±3.77 | 108.11±1.53 | 98.44±1.76 | 91.65±2.13 | |
| G-II +ve control (Glimepride (5mg/kg) | 75.62 ± 3.55^{b} | 103.24±1.55 ^a | 83.42 ± 2.79^{a} | 63.12 ± 1.77^{b} | 60.01 ± 1.67^{b} | 52.57±2.56 ^ª | |
| G-III DLExt 250mg/kg | 79.68±4.77 ^b | 118.08 ± 1.46^{b} | 109.16±2.55 ^b | 103.44±1.65 ^b | 98.52±1.66 ^b | 98.52±2.65 ^b | |
| G-IV DLExt 500mg/kg | 73.35±2.76 ^ª | 111.46±1.67ª | 88.56±4.02 ^b | $70.04 \pm 1.98^{\circ}$ | 66.33±1.56° | $63.67 \pm 2.67^{\circ}$ | |

Table 9: Effect of DLExt of glucose loaded hyperglycemic rats

Tabular values are mean \pm SEM, n = 6; significant difference from control, ${}^{c}p < 0.05$, ${}^{b}p < 0.01$; ${}^{a}p < 0.001$. (OGTT was observed in the Oth, 1st, 2nd, 3rd, 4th& 6th hr), data were analyzed by ANOVA followed by Dunnett test.

| Croups | Blood Glucose level (mg/dl) | | | | | |
|----------------------|-----------------------------|-------------------------|--------------------------|--------------------------|-------------------------|--------------------------|
| Groups | 0hrs. | 1 hrs | 2 hrs | 3 hrs | 4 hrs | 6 hrs |
| G-I -ve control | 82.24±1.55 | 81.74±3.22 | 79.61±1.23 | 78.59± 2.87 | 83.04±2.67 | 79.64±1.65 |
| (Glucose 1.5g/kg) | | | | | | |
| G-II +ve control | 74 41+1 63 | 102 03+1 78 | 82 21+3 67 | 61 91+2 66 | 58 8+2 56 | 51 36+3 53 |
| (Glimepride (5mg/kg) | 71.11±1.05 | 102.05-1.70 | 02.21 - 5.07 | 01.91±2.00 | 50.0-2.50 | 51.50±5.55 |
| G-III | 70.57 ± 1.70^{b} | $42.20\pm1.76^{\circ}$ | $20.04 \pm 2.66^{\circ}$ | 20.05 ± 2.75^{b} | $2802+276^{b}$ | $25.04 \pm 1.76^{\circ}$ |
| DLExt 250mg/kg | 19.57±1.79 | 42.29±1.76 | 39.04-2.66 | 59.05±2.75 | 58.02 <u>1</u> 5.76 | 55.04 <u>1</u> 1.76 |
| G-IV | 78.04 ± 1.88^{b} | 75 23+2 45 ^b | 65 28+2 44 ^b | $59.31 \pm 1.63^{\circ}$ | 45 26+3 52 ^b | $39.06 \pm 1.87^{\circ}$ |
| DLExt 500mg/kg | 70.0T-1.00 | 13.23-2.73 | 03.20-2.77 | 57.51-1.05 | тэ.2013.32 | 37.00-1.07 |

Table 10: Effect of DLExt of normoglycaemic rats

Tabular values are mean \pm SEM, n = 6; significant difference from control, ${}^{c}p < 0.05$, ${}^{b}p < 0.01$; ${}^{a}p < 0.001$. (Hypoglycemic activity was observed in the Oth, 1st, 2nd, 3rd, 4th& 6th hr), data were analyzed by ANOVA followed by Dunnett test

Table11: Effect of DLExt on BGL of alloxan induced diabetic rats.

| Extracts | | Blood Glucose Level (mg/dl) | | | | | |
|-------------------------|-----------------------|-----------------------------|--------------------------|--------------------------|--|--|--|
| Extracts | 0 day | 5 th day | 15 th day | 21 st day | | | |
| G-I (Control) | 77.09 ± 2.43 | 76.02 ± 1.66 | 76.99 ± 1.86 | 78.99 ± 1.77 | | | |
| G-II (Diabetic control) | 288.69 ± 2.67 | 291.41 ± 1.69 | 290.11 ± 2.72 | 289.07 ± 1.88 | | | |
| G-III (Standard) | 266.3 ± 1.67^{a} | 104.13 ± 1.64^{a} | $98.42 \pm 2.67^{\circ}$ | $85.99 \pm 1.84^{\circ}$ | | | |
| G-IV DLExt 500mg/kg | 261.13 ± 1.98^{b} | 144.13 ± 1.54^{b} | 122.02 ± 1.57^{b} | 104.11 ± 1.98^{b} | | | |

Tabular values are mean \pm SEM, n = 6; significant difference from control, ${}^{c}p < 0.05$, ${}^{b}p < 0.01$; ${}^{a}p < 0.001$. (Antidiabetic activity was observed in the 0th, 5th, 15th, 21st day), data were analyzed by ANOVA followed by Dunnett test

3.3.5. Effect of DLExt on body weight of alloxan induced diabetic rats

The loss of body weight in diabetic control animals may be due to increased catabolism of glycogen in muscle and liver, which may be utilized for energy expenditure instead of being stored.

During 21 days of experimental period the G-I gained in the body weight. On the other hand G-II showed reduction in body weight over the same period of time. G-III increased body weight as compared to control. Hydroalcoholic extract of bark of *Dalbergia latifolia* significantly increased body weight in comparison with control group. The ability of Hydroalcoholic extract of bark of *Dalbergia latifolia*, DLExt to protect massive body weight loss seems to be due to is ability to reduce hyperglycemia. The results are summarized in table 12.

| Table 12: Effect of DLExt o | n body | v weight of | falloxan | induced | diabetic ra | its |
|-----------------------------|--------|-------------|----------|---------|-------------|-----|
| | | 67 | | | | |

| Croups | Body weight (gm) | | | | |
|-------------------------|-----------------------|-----------------------|--------------------------|--|--|
| Groups | O th Day | 21 st Day | Relative weight Gain (%) | | |
| G-I (Control) | 158.89±3.53 | 172.99±1.56 | 8.15 | | |
| G-II (Diabetic control) | 166.99 ± 2.45 | 140.19±3.94 | -19.12 | | |
| G-III (Standard) | 169.59±2.67 ° | 177.99±2.65 ° | 4.72 | | |
| G-IV DLExt 500mg/kg | 171.29 ± 1.87^{b} | 175.19 ± 2.67^{b} | 2.23 | | |

Tabular values are mean \pm SEM, n = 6; significant difference from control, ${}^{c}p < 0.05$, ${}^{b}p < 0.01$; ${}^{a}p < 0.001$. (Body weight was observed in the 0^{th} and 21^{st} day), data were analyzed by ANOVA followed by Dunnett test.

3.3.6. Effect of DLExt on serum insulin level of alloxan induced diabetic rats

Sulponyl ureas such as glibenclamide stimulate insulin secretion from pancreatic β cells principally by inhibiting ATP-sensitive K⁺ channels (Watkins 2003). In G-II insulin level reduces by destruction of pancreatic beta cells. The daily treatment of hydroalcoholic extract of bark of *Dalbergia latifolia* exhibited significant increased insulin level could be because of the insulintropic substances present in the extract that induce the protection of the functional or regeneration of β cells so that they remain active and produce insulin. The results are summarized in table 13.

Table 13: Effect of DLExt on serum insulin levelof alloxan induced diabetic rats

| Groups | Serum insulin (µIU/ml) |
|-------------------------|-------------------------|
| G-I (Control) | 30.43 ± 1.45 |
| G-II (Diabetic control) | 11.39±2.48 |
| G-III (Standard) | 28.4 ± 1.09^{a} |
| G-IV DLExt 500mg/kg | 21.22±2.54 ^b |

Tabular values are mean \pm SEM, n = 6; significant difference from control, ${}^{c}p < 0.05$, ${}^{b}p < 0.01$; ${}^{a}p < 0.001$. (Serum insulin was observed on the 21st day), data were analyzed by ANOVA followed by Dunnett test

3.3.7. Effect of DLExt on serum lipids level of alloxan induced diabetic rats

The level of serum lipids are usually elevated in diabetes mellitus. The abnormal high concentration of serum

lipids in diabetes is mainly due to increasing mobilization of free fatty acids. In alloxan induced diabetic rats, the marked hyperglycemia is accompanied by an increase in serum cholesterol and triglycerides. G-II exhibited marked abnormalities in lipid metabolism as evidenced by elevation of serum TG, TC, LDL, VLDL and reduction of HDL level, whereas in G-III significant lipid lowering effect was observed. The regular administration of G-IV for 21 days nearly normalized lipids level in diabetic animal through the control of hyperglycaemia. Treatment was sufficient to produce reduction in level of TG, TC, LDL, VLDL and elevation in HDL level in diabetic rats. Decreased serum lipid level might have induced by increased insulin release. Results of serum lipid profile is summarized in table 14.

3.3.8. Effect of DLExt on lipid peroxidation of alloxan induced diabetic rats

Increased lipid peroxidation was observed because of increased oxidative stress in the cell that may impairs membrane function through the reduction of membrane fluidity and also by changing the activity of membrane bound enzymes as well as receptors. The study implies that average concentrations of MDA were significantly increased in diabetic rats when compared with the control group. Natural extracts with antioxidant activity might totally or partially alleviate damage. Therefore group treated with G-IV significantly decreased MDA in pancreas and in kidney indicated decreased oxidative stress (table 15).

| Croups | Lipid Profile | | | | | |
|-------------------------|--------------------------|--------------------------|-------------------------|----------------------|-------------------------|--|
| Groups | TG (mg/dl) | TC (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | |
| G-I (Control) | 80.38±2.45 | 76.08±1.66 | 47.28±3.77 | 17.98±3.66 | 15.98±3.01 | |
| G-II (Diabetic control) | 153.48±1.45 | 119.28±2.55 | 30.58 ± 2.87 | 44.88±2.76 | 28.08 ± 2.67 | |
| G-III (Standard) | 110.18 ± 2.89^{a} | 82.18±3.77ª | 40.38±3.01 ^a | 29.28±1.76ª | 20.38 ± 2.77^{a} | |
| G-IV DLExt 500mg/kg | 125.58±3.86 ^b | 104.98±2.66 ^b | 35.18±3.98 ^b | 38.48 ± 2.66^{a} | 24.28±2.66 ^b | |

Table 14: Effect of DLExt on serum lipids level of alloxan induced diabetic rats

Tabular values are mean \pm SEM, n = 6; significant difference from control, ^b p < 0.01; ^a p < 0.001. (Serum lipids level was observed on the 21st day), data were analyzed by ANOVA followed by Dunnett test.

| Table15: Effect of DLExt on li | pid | peroxidation | of alloxan | induced | diabetic rat | S |
|--------------------------------|-----|--------------|------------|---------|--------------|---|
|--------------------------------|-----|--------------|------------|---------|--------------|---|

| Groups | Pancreas TBARS (nmol MDA/ mg protein) | Kidney TBARS (nmol MDA/ mg protein) |
|-------------------------|--|--|
| G-I (Control) | 0.359 ± 1.62 | 0.512 ± 2.76 |
| G-II (Diabetic control) | 2.346 ± 1.67 | 3.79±3.88 |
| G-III (Standard) | 0.97 ± 1.56 | 1.28 ± 2.64^{a} |
| G-IV, DLExt 500mg/kg | 1.82 ± 1.34 | 2.78±1.98 ^b |

Tabular values are mean \pm SEM, n = 6; significant difference from control, ${}^{c}p < 0.05$, ${}^{b}p < 0.01$; ${}^{a}p < 0.001$. (Pancreas and kidney TBARS was observed on the 21st day), data were analyzed by ANOVA followed by Dunnett test

3.3.9. Effect of DLExt on glycogen level of alloxan induced diabetic rats

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues are direct reflection of insulin activity. Insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since alloxan damages beta cells resulting in decrease in insulin level and glycogen levels decrease as it depend on insulin influx of glucose. In diabetic condition glycogen level stored in liver and skeletal muscle is reduced. Treatment with G-II increased glycogen content due to glycogen storage. In response to enhanced insulin levels the glycogen content in liver was found to increase which was significant G-IV as compared to control. The results are summarized in table 16.

3.3.10. Effect of DLExt on total antioxidant enzymes of alloxan induced diabetic rats

Persistent hyperglycemia leads to increased production

of free radicals. The antioxidant enzymes are inhibited in DM as a result of non enzymatic glycosylation and oxidation. Treatment with G-IV significantly increased SOD, CAT, GPx and GST activity as compared with diabetic group. GSH level was observed in G-IV. The result implies that DLExt may reduce oxidative stress by increasing antioxidant defense due to presence of several bioactive antioxidant principles. Results are summarized in table 17.

Table 16: Effect of DLExt on glycogen level ofalloxan induced diabetic rats

| Groups | Liver glycogen (mg/g) |
|-------------------------|-----------------------|
| G-I (Control) | 54.82 ± 1.56 |
| G-II (Diabetic control) | 17.67±2.54 |
| G-III (Standard) | 39.71 ± 1.77^{a} |
| G-IV, DLExt 500mg/kg | 34.59 ± 2.98^{b} |

Tabular values are mean \pm SEM, n = 6; significant difference from control, ${}^{c}p < 0.05$, ${}^{b}p < 0.01$; ${}^{a}p < 0.001$. (Glycogen level was observed on the 21st day), data were analyzed by ANOVA followed by Dunnett test.

| Table 17: Effect of DLExt on total antioxidant enzy | symes of alloxan induced diabetic rats |
|---|--|
|---|--|

| Croups | Total Antioxidant Enzymes (U mg ⁻¹ protein) | | | | | |
|-------------------------|--|-------------------------|------------------------|------------------------|-------------------------|--|
| Groups | SOD | CAT | GPx | GST | GSH | |
| G-I (Control) | 3.83±1.21 | 71.1±1.34 | 3.84±2.63 | 3.94±2.56 | 42.88±1.56 | |
| G-II (Diabetic control) | 1.27 ± 3.67 | 47.07±1.76 | 1.07 ± 1.32 | 1.37±1.99 | 20.67 ± 1.45 | |
| G-III (Standard) | 3.66±3.12° | 58.07 ± 1.66^{a} | 3.27±1.67 ^b | 3.07±1.77 ^b | 39.07±1.65 ^b | |
| G-IV DLExt 500mg/kg | 2.13±2.65 ° | 50.89±1.54 ^b | 1.81±1.87 ^b | 2.45±1.55 ^b | 32.26±1.98 ^b | |

Tabular values are mean \pm SEM, n = 6; significant difference from control, ^b p < 0.01; ^a p < 0.001. (Total antioxidant enzymes was observed on the 21st day), data were analyzed by ANOVA followed by Dunnett test

3.3.11. Histopathological studies

Comparative histological evaluation is a good parameter for understanding the various extracts individual potential to alter the histo architecture with respect to control and alloxan treated group. For histopathological studies, paraffin sections (5 μ m) of the pancreatic tissues were studied for ultra structural changes. The histopathological studies revealed rapid uptake of insulin secreting cells by alloxan displaying extensive necrosis, degranulation and hydrotropic change. Liver and other cells are more resistant to ROS in comparison to pancreatic β cells and this resistance protects them against alloxan toxicity.

3.3.11.1. Histopathological studies of pancreas

The histology of pancreatic islet cells control rat showed normal architecture with islet of langerhans which formed of numerous compactly arranged cells (Photograph). Histological, in diabetic rats with no treatment, consistent findings in section of pancreatic cells were significant changes in the morphology of pancreatic cells including mild swelling and inflammation, β cell degranulation, decreased cellular density and indistinct border between endocrine and exocrine region (Photograph). Diabetic rats treated with DLExt showed minimal to moderate degree of necrotic and fibrotic changes and atrophy of islets of Langerhans (Photograph). In diabetic rats treated with DLExt there was remarkable improvement in pancreatic tissues with distinct cellularity changes and viable islets cells (Photograph 1, 2, 3).



Photograph 1: Photomicrograph of pancreas section of animal treated with vehicle (Control)



Photograph 2: Photomicrograph of pancreas section of animal treated with alloxan (Diabetic control)



Photograph 3: Photomicrograph of pancreas section of animal treated with hydroalcoholic extract of bark of *Dalbergia latifolia*.

3.3.11.2. Histopathological studies of liver

Histopathological studies of liver cells of animals treated with various extracts were performed to study the changes in histoarchitecture. The photomicrograph of control rats showed normal hepatocytes with well brought out nuclei and cytoplasm (Photograph). Histological examination of liver of the diabetic rats showed hydrophobic degeneration and disruption of remarc cordons in hepatocytes specifically localized around the lobes of liver (Photograph). The rats treated with DLExt showed hepatocytes with nearly normal appearance and minimal necrosis (Photograph). Rats treated with DLExt showed normal architecture of hepatocytes by significantly reduced degree of diabetic induced necrosis in hepatocytes (Photograph 4, 5, 6).



Photograph 4: Photomicrograph of liver section of animal treated with vehicle (Control)



Photograph 5: Photomicrograph of liver section of animal treated with alloxan (Diabetic control)



Photograph 6: Photomicrograph of liver section of animal treated with hydroalcoholic extract of bark of *Dalbergia latifolia*.

3.3.11.3. Histopathological studies of kidney

Histopathological studies of kidney cells of animals treated with alcoholic and aqueous bark extracts were performed to study the changes in histoarchitecture. The photomicrograph of control rat showed normal architecture with normal bowman's capsule and renal tubules (Photograph). Histological examination of kidney of the alloxan induced diabetic rats showed significant damage in renal structure showing marked glomeruli and tubular damages, due probably to the generation of reactive radicals and to subsequent lipid peroxidation induced by alloxan (Photograph).



Photograph 7: Photomicrograph of kidney section of animal treated with vehicle (Control)



Photograph 8: Photomicrograph of kidney section of animal treated with alloxan (Diabetic control)

The diabetic rats treated with DLExt showed minimal to moderate degree of changes in glomerulus with

reduction in thickening of walls of renal tubules (Photograph). The diabetic rats treated with DLExt showed normal architecture of bowman's capsule and renal tubules by improved the histological alterations induced by alloxan (Photograph 7,8,9).



Photograph 9: Photomicrograph of kidney section of animal treated with hydroalcoholic extract of bark of *Dalbergia latifolia*

4. CONCLUSION

Traditionally *Dalbergia latifolia* is used in treatment of diarrhoea, indigestion, antipyretic, expectorant, appetizer, anthelmentic, diabetes and cure skin disease etc. *Dalbergia Latifolia* Roxb also has one special feature which is used in enriching soil environment because it has capability of Nitrogen-fixation. The present study was carried on anti diabetic potential of *Dalbergia Latifolia* Roxb and its effect on lipid profile and hepatic enzymes of glucose metabolism in diabetic rats.

The results suggested that DLExt (500mg/kg) induced a potent reduction in glycaemia with maximum fall may be due to increase in insulin concentration in glucose loaded rats. DLExt (500mg/kg) exhibited maximum potential to reduce the BGL. The possible mechanism by which the plant extract mediates antidiabetic action might be potentiation of pancreatic secretion of insulin from existing residual β cell of islets or due to enhanced transport of blood glucose to peripheral. The ability of Hydroalcoholic extract of bark of Dalbergia latifolia, DLExt to protect massive body weight loss seems to be due to is ability to reduce hyperglycemia. Effect of DLExt on total antioxidant enzymes of alloxan induced diabetic rats was also studied and DLExt significantly decreased MDA in pancreas and in kidney indicated decreased oxidative stress. Treatment with DLExt

significantly increased SOD, CAT, GPx and GST activity as compared with diabetic group. The result implies that DLExt may reduce oxidative stress by increasing antioxidant defense due to presence of several bioactive antioxidant principles. Treatment was sufficient to produce reduction in level of TG, TC, LDL, VLDL and elevation in HDL level in diabetic rats. The histopathology studies suggested that diabetic rats treated with DLExt there was remarkable improvement in pancreatic tissues with distinct cellularity changes and viable islets cells. Rats treated with DLExt showed normal architecture of hepatocytes by significantly reduced degree of diabetic induced necrosis in hepatocytes. The diabetic rats treated with DLExt showed normal architecture of bowman's capsule and renal tubules by improved the histological alterations induced by alloxan.

The results of the study clearly suggest the promising potential of plant in the treatment and management of diabetes and can be used as potential candidate in the preparation of anti diabetic formulations. The study can further be carried out in the direction of chemical characterization of the phytochemical responsible for the in order to generate complete profile of *Dalbergia latifolia*.

Conflict of Interest

The author (s) declares that there is no conflict of interest.

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