

Evaluations of antidiabetic potential of the hydro-alcoholic extract of the stem bark of *Plumeria rubra* a traditionally used medicinal source in North-East India

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Abstract

Aim: Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. Its complications such as neuropathy, cardiopathy, nephropathy, and micro- and macro-vascular diseases are believed to be due to the increase in oxidative stress. The present study aimed to evaluate the hypoglycemic activity of hydro-ethanolic (3:7) stem bark extracts of *Plumeria rubra* (Family: Apocynaceae) to streptozotocin-induced diabetic rats. **Materials and Methods:** The standardized doses of 250 and 500 mg/kg body weight of the extract were administered orally to normal and diabetic rats to define its hypoglycemic potential. The blood glucose levels were measured using single touch glucose test strips and glucometer on weekly intervals until the end of the 21st day. Other parameters such as lipid profiles and liver enzymes were also investigated after the treatment periods. **Results and Discussion:** Results of the present investigation suggest that diabetes is associated with an increase in oxidative stress as shown by the increase in serum malondialdehyde, decreased levels of catalase, and glutathione. Furthermore, diabetes is associated with an increase in serum total cholesterol as well as triglycerides and alkaline phosphatase levels. The histopathological changes of the diabetic and vehicle control animals were also investigated here. **Conclusion:** Daily oral administration of hydro-alcoholic stem bark extracts and metformin hydrochloride as standard drug (5 mg/kg body weight) showed improvement effect of lowering blood glucose level as well as hyperlipidemia and liver functions. The extract has beneficial to maintain the animal body weight and has the regenerating power on pancreatic cells.

Key words: Antidiabetic, glucose tolerance test, hypoglycemia, *Plumeria rubra*

INTRODUCTION

Diabetes mellitus (DM) has affected a considerable population, and in the future, it will be a major disorder, affecting people across the globe, irrespective of sex, age, and socioeconomic status. Diabetes mellitus is an endocrine disorder that is characterized by chronic hyperglycemia.^[1] The prevalence of diabetes is rising all over the world due to population growth, aging, urbanization, and an increase of obesity and physical inactivity. Unlike in the West, where older persons are most affected, diabetes in Asian countries is disproportionately high in young to middle-aged adults. This could have long-lasting adverse effects on a nation's health and economy, especially for developing countries.

Insulin is a protein hormone secreted by the beta cells of islets of Langerhans of the pancreas. Deficiency of effective insulin in the body causes diabetes mellitus.^[2] Type I diabetes (Insulin dependent) is caused due to insulin insufficiency because of lack of functional beta cells. Patients suffering from Type I are, therefore, totally dependent on exogenous source of insulin, whereas patients suffering from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise, and

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medication.^[3,4] Genetic predisposition is associated with Type I diabetics and it represents only 5% of total diabetic patients although in case of Type II DM (nondependent insulin diabetic mellitus), there is the lack of endogenous insulin in the circulation. Multiple biochemical impairments associate with micro- and macro-vascular complications, which is a major cause of morbidity and death in DM.^[5,6] With the numbers of people affected by diabetes multiplying worldwide, the disease is taking an ever increasing proportion of national and international health-care strategies.^[7] Diabetes mellitus occurs throughout the world; however, it is more common in the developing and developed countries. Diabetes is in the top 10, perhaps in the top 5, of the most significant diseases in the developed world and is still gaining significance.^[8] The World Health Organization (WHO) has predicted that the major burden will occur in developing countries. India has today become the diabetic capital of the world. Studies conducted in India in the last decade have highlighted that not only is the prevalence of diabetes high but also that it is increasing rapidly in the urban populations. It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025.^[9] The International Diabetes Federation and also the WHO estimate that worldwide over 100 million people suffer from type II diabetes and 50% of those cases are undiagnosed.

Since the discovery of insulin, several synthetic oral hypoglycemic drugs are available in the market. These modern medicines exert serious side effects such as hepatotoxicity, abdominal pain, diarrhea, and hypoglycemia.^[10,11] Oral hypoglycemic agents and clinical grade insulin are synthetic ones having certain serious adverse side effects as well not suitable for use during pregnancy. Thus, promoting alternative therapeutic approaches and to find safer hypoglycemic agents, the ethnopharmacological knowledge of plants which have proved beneficial due to their effectiveness, safety, lower toxicity, and fewer side effects compared to synthetic drugs and age old practices as folklore medicine have become important.^[12] However, there is an ever increasing demand for complementary and alternative medicines with higher achievable antihyperglycemic principles.^[13] In general, the phytochemicals have fewer side effects. Many of them are helpful in preventing the secondary complications of diabetes.^[14] Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to serve mankind with new remedies. At present, there is a worldwide movement or assessing the plant resources, which are of medicinal and economical value and importance. Researchers are focusing mainly on ethnobotanical and ethnomedicinal investigations to fulfill the increasing demand of herbal products. The WHO has also recommended the evaluation of traditional plant treatments for diabetes as they are effective, nontoxic, with less or no side effects and are considered to cost effective.^[15] The WHO has listed 21,000 plants, which are being used for medicinal purposes around the world.^[16] WHO also estimates that about

80% of the population in the developing countries directly depends on plants for its medicines^[17,18] and more than 2500 medicinal plant species and 2000 medicinal preparations are in India.^[19]

The plant *Plumeria rubra* is a roadside tree of the north-eastern region of India belonging to the family Apocynaceae and commonly known as “Gour-Champa [Figure 1].” The old tree (9-10 years) is 20-40 feet in height and covered with gray bark. The different parts of the trees have multidisciplinary aspect to human. The plant material is widely used as purgative, remedy for pain, fever, diarrhea, and cure for itch. The milky juice is employed for the treatment of inflammation. The essential oil from the flowers possesses antifungal activity.^[20] The traditional use of *P. rubra* barks as an antidiabetic agent, together with modern pharmacological studies, suggested it has potential therapeutic effects. However, no previous studies have focused on the *in vivo* antidiabetic activity of this medicinal bark. Therefore, the present study was conducted to investigate the efficacy of the hydro-alcoholic stem bark extract of *P. rubra* as an antidiabetic agent and explore its potential mechanism on streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Chemicals and Solvents

STZ was purchased from Sigma Chemical Co., USA. Metformin hydrochloride was gifted from East and West Pharmaceutical, Roorkee, India. The cholesterol, triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) kits were purchased from Span Diagnostics, Surat, India. While serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) kits were bought from Medsource Ozone Biomedicals, India.

Plant Material

The stem barks of *P. rubra* (Family - Apocynaceae) were collected from garden of Dibrugarh University Campus, Assam. The Botanical survey of India, Shillong, identified the plant taxonomically. A voucher specimen (DU/PM/2012/8) of the collected sample deposited in the institutional herbarium and departmental museum for future references.

Preparation of Extract

The freshly collected stem barks of *P. rubra* were air-dried for 25 to 30 days at room temperature under the control condition, reduced to coarse powder through sieve no. 40 and stored in an air-tight container. The powdered bark was extracted with ethanol and water (3:7, most of the flavonoids

and the glycosides present in this hydro-alcohol mixture) by cold maceration process. The cold maceration process has the less chance to degrade the thermolabile components (flavonoids and glycosides) over the continuous heat process extraction as using Soxhlets apparatus. The extract was then filtered and evaporated to dryness at - 40°C under reduce pressure in a lyophilizer. The dark brown mass was stored in desiccators for uses.

Animal Care and Maintenance

Experiments were performed with 6-8 weeks old, healthy, male albino Wistar rats, of body weight 150-250 g. Rats were housed under standard environmental conditions (25 ± 2°C temperature, 50 ± 5% humidity with a 12:12 h dark and light cycle) and maintained with free access to water and a standard pellet (Lipton India, Ltd.). The animals were used with the approval of the Institute Animal Ethics Committee (Approval no. IAEC/DU/37 Dated 03/12/2012. Regd. No. 1576/Go/a/11/CPCSEA dated 17.02.2012). Animals described as fasted were deprived of food for 16 h but had free access to water.

Acute Oral Toxicity Study

The Wistar albino rats were used for the oral toxicity test, and the animals were received the plant extract after overnight fasting. The oral toxicity was performed as the Organization for Economic Corporation and Development guidelines 425.^[21] After administration of plant extract, food was withheld for further 3-4 h. Animals were observed individually at least once during 30 min after dosing, periodically during the first 24 h. (with special attention during first 4 h) then daily up to 14 days. At the end of the study, the animals were observed for general toxic signs, morphological behaviour, and mortality. One-tenth of LD₅₀ (>2500 mg/kg body weight) was taken for further study (Organization for Economic Cooperation and Development, 2006).

Experimental Design

The 0.5% carboxymethyl cellulose (CMC) solution was used as vehicle. The drugs were administered to animals orally using an intragastric tube daily for 3 weeks. Overnight fasted normal animals were randomly divided into five groups of six rats in each group.

The Group I: Served as vehicle control, which received vehicle, i.e., 0.5% CMC solution.

Group II: Diabetic control which received STZ (55mg/kg body weight).

Group III: Standard group: STZ-induced diabetic rats received metformin hydrochloride 5 mg/kg body weight orally.^[22]

Group IV: STZ-induced diabetic rats were treated with hydro-alcoholic stem bark extract (HSBE) at 250 mg/kg body weight orally.

Group V: STZ-induced diabetic rats were treated orally with HSBE at 500 mg/kg body weight.

Fasting blood glucose (FBG) was determined with glucometer and single touch strips.

Oral Hypoglycemic Effect of Extracts in Normal Rats

The oral glucose tolerance test (OGTT) performed on overnight fasted normal animals. Rats divided into four groups were administered 0.5% CMC solution, metformin hydrochloride 5 mg/kg, and HSBE (250 mg/kg and 500 mg/kg) dissolved in vehicle, respectively. Glucose (3 g/kg) was feed 30 min after the administration of samples. Blood was withdrawn from puncture of tail vein at 0, 30, 60, 90, and 120 min of sample administration. Blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips.^[23] In this experiment, we have used metformin hydrochloride as a standard drug. Metformin hydrochloride improves hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis). Metformin activates AMP-activated protein kinase (AMPK), an enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats, activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells.^[24]

Induction of Diabetes and Blood Glucose Estimation

Overnight fasted albino rats were made diabetic by injecting STZ intraperitoneally at a dose of 55 mg/kg body weight.^[25]

STZ weighted individually for each animal according to the weight and then solubilized with 0.2 ml normal saline just before injection. Diabetes was confirmed in STZ-injected rats by measuring the FBG concentration after 72 h. Rats with blood glucose level above 250 mg/dl were considered diabetic and were used in this study. Blood samples were then collected for determination of blood glucose level on 0, 7, 14, and 21 days.

Biochemical Estimation

At the end of 21st day, after the estimation of blood glucose level, the animals were sacrificed. Blood was collected by cardiac puncture and serum was separated for determination of biochemical parameters, the level of total cholesterol (TC), serum TG, HDL, and LDL were estimated using commercial available kits (SPAN diagnostics kit). The SGOT, SGPT, ALP and lipid peroxidase, catalase (CAT), and glutathione (GSH) activity also measured for the assessment of antidiabetic activity.

Tissue Sample Preparation for Measurement of Enzymatic Antioxidants

The animals were sacrificed by cervical dislocation. Liver was excised, cleaned of gross adventitial tissue, blotted dry and processed for biochemical measurements (CAT, GSH, and malondialdehyde [MDA]). Tissues were homogenized in 5.0% (w/v) 0.15 M KCl and centrifuged at 800× g for 10 min. The cell-free resultant supernatant was used for the measurement of enzymatic and nonenzymatic antioxidants.^[26]

Lipid Peroxidation (LPO)

LPO was assayed by the measurement of MDA levels on the basis of reaction with thiobarbituric acid.^[27] Briefly, 0.2 ml of serum was mixed with thiobarbituric acid reagent (0.375% thiobarbituric acid and 15% tri-chloro-acetic acid in 0.25 N HCl). The reaction mixture of serum and thiobarbituric acid reagent was placed in boiling water for 15 min, cooled, centrifuged, and then, the optical density of the supernatant was recorded at 532 nm.

Estimation of Reduced GSH

The GSH level was estimated according to the method of Moron *et al.*^[28] The protein-free filtrate after precipitation with metaphosphoric acid was reacted with 5-5 di-thio-bis (2-nitrobenzoic acid) (CDNB). The CDNB and sulfhydryl groups form a relatively stable yellow that can be read at 420 nm wavelength against the blank.

Estimation of CAT

CAT activity was estimated by determining the decomposition of H₂O₂ at 240 nm in an assay mixture containing phosphate buffer.^[29] One international unit of CAT utilized is that amount that catalyzes the decomposition of 1 mM H₂O₂/min/mg of protein at 37°C. CAT activity was calculated using the mill molar extinction coefficient of 0.07 and expressed in terms of micromole per minute per milligram of protein.

Histopathological Analysis

The experiment has performed the histopathological study of rats' pancreas of control as well as extract treated. The pancreas was washed in saline and a small portion of these organs was quickly fixed in 10% of formalin solution. Then, the tissues were processed by standard histopathological technique (i.e., dehydration through graded isopropyl alcohol, cleaning through xylene, and impregnated in paraffin wax for 2 h). Wax blocks were made, sections were used for cutting microtome and stained by hematoxylin eosin (H and E) method.^[30]

Statistical Analysis

All result were expressed as the mean±standard error of mean (SEM). The results were analyzed for statistical significance by One-way analysis of variance followed by Dunnett's multiple comparison tests. The significance was expressed by "P value," as mention in the tables. $P < 0.01$ was considered as statistically significant.

RESULTS AND DISCUSSION

Diabetes is one of the leading diseases around the globe. Management of diabetes is being a tough task with the synthetic medicines as they have many side effects. The interest has been increased on the medicinal plants used for remedy or reducing the risk of diseases. In the recent scenario, the scientists have emphasized for the herbal extracts and initiated extensive research to observe their effective and protective role in the diseased animal models. The OGTT showed the blood glucose levels of control group reach a peak decreased at 30 min and continuously decrease to attain basal glucose level. Both fractions of extract of plant (250 mg/Kg and 500 mg/Kg) decreased blood glucose level gradually at 90 and 120 min [Table 1].

In this experiment, we used the STZ for inducing of diabetes to rats. STZ is a preferred agent to induce experimental diabetes since it has some advantages over alloxan such as relatively longer half-life (>15 min), sustained hyperglycemia for longer duration, and the development of well-characterized diabetic complications with fewer incidences of ketosis as well as mortality.^[31] STZ acts as a diabetic agent by the destruction of β-cells of pancreatic islets of Langerhans and the resultant reduction of insulin release. Insulin deficiency leads to various metabolic alterations in the animals, viz., increased blood glucose, increased cholesterol, increased level of alkaline phosphate and transaminases, etc.^[32,33]

The present results indicate that significant increase in body weight and decrease in blood glucose levels in diabetic rats throughout the 21 days of study, and they became normal when treated with the plant extracts. A significant increase of body weight was noted from day 1st to 21st days, i.e., 233.16 g to 237.33 g and 232.00 g to 239.16 g in case of HSBE 250 mg/kg and 500 mg/kg body weight, respectively [Table 2]. On the other side, the gradual fall of blood glucose value was observed from 246.04 mg/dl to 134.33 mg/dl and 244.12 mg/dl to 123.33 mg/dl, respectively, in the duration of 1st day to 21st days [Table 3].

The fundamental mechanism underlying hyperglycemia involved over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues.^[34]

Table 1: Report for the OGTT

Animal group	0 min	30 min	60 min	90 min	120 min
G-I vehicle control	78.16±1.22	140.50±4.32 [#]	152.16±2.12 [#]	167.00±1.62 ^{##}	186.51±1.2 [#]
G-II HSBE (250 mg/kg)	80.32±3.16	137.10±1.35	154.32±1.13 ^{**}	126.12±2.34 ^{###}	122.22±1.44 ^{####}
G-II HSBE (500 mg/kg)	79.12±2.23	135.24±2.43	149.50±4.25 ^{**}	112.23±3.46 ^{###}	94.00±2.31 ^{####}
G-IV standard drug (5 mg/kg)	80.15±1.51	128.15±3.21 [*]	118.20±1.86 [*]	101.32±1.62 ^{**}	86.21±1.53 ^{**}

Values are mean±SEM (n=6), statistical significance: *P<0.05, **P<0.01 compared with control group I; #P<0.05, ##P<0.01 compared with standard drug group IV. OGTT: Oral glucose tolerance test, SEM: Standard error of mean

Table 2: The deviation of body weight of the animals during the treatment of HSBE of *P. rubra* during 21 days of treatment

Treatment	0 day	7 th day	14 th day	21 st day
Normal control (Group-I)	230.83±0.91 ^{**}	221.13±2.56ns	238.33±0.39	247.16±0.69 ^{**}
Diabetic control (55mg/kg STZ) (Group-II)	222.50±0.54 ^{##}	225.19±1.20ns	193.16±1.19 [#]	183.66±0.89 ^{##}
HSBE (250 mg/kg) (Group-III)	233.16±0.42 ^{##}	231.23±2.31ns	230.16±0.85 ^{**}	237.33±0.86 ^{###}
HSBE (500 mg/kg) (Group-IV)	232.00±0.60 ^{##}	219.49±1.53ns	234.50±0.41 ^{###}	239.00±0.48 ^{###}
Standard drug (Group-V)	227.50±0.73 ^{**}	210.24±1.32ns	228.66±0.63 ^{**}	232.83±0.55 ^{**}

Values are mean±SEM (n=6), statistical significance, *P<0.05, **P<0.01 compared with normal control Group I; #P<0.05, ##P<0.01 compared with diabetic control Group II and ns – nonsignificant. SEM: Standard error of mean, HSBE: Hydro-alcoholic stem bark extract, *P. rubra*: *Plumeria rubra*

Table 3: The effect of HSBE of *P. rubra* on fasting blood glucose level on streptozotocin-induced diabetic rats

Treatment	0 day	7 th day	14 th day	21 st day
Normal control (Group-I)	92.53±1.87	103.36±3.31 ^{##}	101.12±4.29 ^{##}	98.15±2.23 ^{##}
Diabetic control (Group- II)	245.23±3.31	262.00±4.34 ^{**}	265.13±7.20 ^{**}	271.23±5.30 ^{**}
Standard drug (Group-III)	240.54±4.06	160.45±2.30 ^{###}	135.13±6.40 ^{###}	116.12±8.77 ^{**}
HSBE (250 mg/kg) (Group-IV)	246.04±4.20	184.14±5.40 ^{###}	176.32±3.30 ^{###}	134.33±3.50 ^{###}
HSBE (500 mg/kg) (Group-V)	244.12±5.80	162.20±6.20 ^{###}	138.42±7.20 ^{###}	123.33±3.54 ^{###}

Values are mean±SEM (n=6), statistical significance, *P<0.05, **P<0.01 compared with normal control Group I; #P<0.05, ##P<0.01 compared with diabetic control Group II. SEM: Standard error of mean, HSBE: Hydro-alcoholic stem bark extract, *P. rubra*: *Plumeria rubra*

The levels of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents a risk factor for coronary heart disease. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities.^[35-38] The increased level of lipid TC and TG in streptozotocinized rats observed in this experiment. Regular administration of HSBE not only leads to normalized the lipid profile of diabetic rats but also enhanced the HDL cholesterol which is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed “reverse cholesterol transport,” and is considered to be a cardioprotective lipid. A highly negative correlation between serum HDL-cholesterol level and incidences of atherosclerosis^[39] is also known to exist. Thus, HSBE has a significant role to improving the imbalance in lipoprotein metabolism [Table 4].

Liver enzymes are liberated into blood whenever liver cells are damaged, and enzyme activity in the plasma is increased. Increased activity of transaminases, which are active in the absence of insulin because of increased availability of amino acids in diabetes, are believed responsible for the increased gluconeogenesis and ketogenesis observed in the disease.^[40] Alanine and aspartate transaminase activities are used as an indicator of hepatocyte damage. Acid phosphatase activity is normally high in diseased states and is often used as a tool in the clinical investigation. Data from this study show that alanine transaminase activity is elevated in diabetes, whereas the feeding of the extracts resulted in a significant decrease in alanine transaminase [Table 5].

Many studies showed that hyperglycemia is among the contributing factors involved in most diabetic complications through excessive production of reactive oxygen species.^[41] Oxidative damage has been suggested to be a contributory factor in the development and complications of diabetes. LPO, a type of oxidative degeneration of polyunsaturated lipids, has been implicated in a variety of pathogenic

processes.^[42,43] The elevated levels of blood glucose in diabetes produce oxygen-free radicals that cause membrane damage due to peroxidation of membrane lipids and protein glycation. The oxidative stress and resultant tissue damage are an important component in the pathogenesis of diabetic complications.^[44] By the oxidative stress induced by STZ may lead to an imbalance of *in-vivo* antioxidant system and that was evaluated by this study. The oxidative stress in the diabetic animals measured by markers since the free radical (CH_3^+) measurement is difficult due to their very short half-life and their low concentration. Therefore, indirect markers are commonly used to evaluate secondary products of LPO such as thiobarbituric acid reactive species.^[45] Elevated lipid peroxidation was also studied in STZ diabetic animal. HSBE and metformin hydrochloride treated groups were significantly reduced the oxidation of lipids, the data represented on Table 6.

GSH plays the important role in balance the oxidative stress. In diabetic control groups, the decreased GSH may be due to a reduction in GSH synthesis or degradation of GSH by oxidation stress in the diabetic animal. Previous studies have reported that there was an increased LPO in liver and kidney of diabetic rats.^[46] Under *in-vivo* conditions, GSH acts as an antioxidant and its decrease was reported in diabetes mellitus.^[44] Here, observed a significant decreased in GSH levels during diabetes. The decrease in GSH levels represents increased utilization due to oxidative stress.^[47] The depletion of GSH content may also lower the GST activity as GSH is required as a substrate for GSH *S*-transferases (GST) activity.^[48] The increased GSH content in the liver and kidney of the rats treated with HSBE and metformin may be one factor responsible for inhibition of LPO.

CAT is one of the major scavenging enzymes that remove toxic free radicals *in-vivo*. Reduced activities CAT in liver

Table 4: Effects of HSBE of *P. rubra* on cholesterol, TG, HDL, and LDL of control and experimental rats

Treatment	Cholesterol (mg/dl)	TD (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal control (Group-I)	162.26±4.8 [#]	213.32±6.8 [#]	40.39±2.3 [#]	68.64±3.8 [#]
Diabetic control (Group-II)	230.05±6.5 ^{**}	302.12±4.9 ^{**}	25.93±2.2 ^{**}	136.43±4.2 ^{**}
Standard drug (Group-III)	168.08±6.7 ^{**}	240.02±5.6 ^{***}	34.02±1.9 [*]	85.68±3.8 ^{***}
HSBE (250 mg/kg) (Group-IV)	203.25±5.2 ^{***}	265.33±5.2 ^{***}	28.11±1.5 [#]	107.17±5.1 ^{***}
HSBE (500 mg/kg) (Group-V)	185.12±7.4 ^{***}	240.23±11.3 ^{***}	33.56±1.7 [*]	86.06±5.3 ^{***}

Values are mean±SEM (n=6), statistical significance, *P<0.05, **P<0.01 compared with Normal control Group I; #P<0.05, ##P<0.01 compared with diabetic control Group II. TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, HSBE: Hydro-alcoholic stem bark extract, *P. rubra*: *Plumeria rubra*, SEM: Standard error of mean

Table 5: Effect of HSBE of *P. rubra* on liver enzymes of control and experimental rats

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Normal control (Group-I)	50.46±4.9 [#]	43.21±4.4 [#]	105±2.4 [#]
Diabetic control (Group-II)	100.79±7.2 ^{**}	84.23±3.3 ^{**}	175±3.6 ^{**}
Standard drug (Group-III)	58.38±5.6 ^{**}	49.33±2.8 ^{**}	122±2.8 ^{***}
HSBE (250 mg/kg) (Group-IV)	78.10±4.8 ^{##}	60.13±5.1 ^{***}	138±4.2 ^{**}
HSBE (500 mg/kg) (Group-V)	62.32±5.3 ^{**}	54.32±3.4 ^{**}	127±5.1 ^{***}

Values are mean±EM (n=6), statistical significance, *P<0.05, **P<0.01 compared with normal control Group I; #P<0.05, ##P<0.01 compared with diabetic control Group II. *P. rubra*: *Plumeria rubra*, SEM: Standard error of mean, HSBE: Hydro-alcoholic stem bark extract, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase

Table 6: Antioxidant status of the extract HSBE on experimental rats

Treatment	Peroxidase (nM MDA/mg of protein)	GSH (nM MDA/mg of protein)	CAT (µM of H ₂ O ₂ decomposed/min/mg protein)
Normal control (Group-I)	15.057±0.83 [#]	52.699±0.56 [#]	13.04±0.28 [#]
Diabetic control (Group- II)	35.230±0.52 [*]	21.230±0.32 [*]	5.47±0.84 [*]
Standard drug (Group-III)	28.283±0.56 [#]	35.147±0.26 [#]	8.50±0.53 [#]
HSBE (250 mg/kg) (Group-IV)	26.998±0.22 ^{**}	37.448±0.22 ^{##}	6.80±0.66 [*]
HSBE (500 mg/kg) (Group-V)	23.282±0.35 ^{##}	44.198±0.78 ^{**}	7.60±0.38 ^{##}

Values are mean±SEM (n=6), statistical significance, *P<0.05, **P<0.01 compared with normal control Group I; #P<0.05, ##P<0.01 compared with diabetic control Group II. SEM: Standard error of mean, HSBE: Hydro-alcoholic stem bark extract, MDA: Malondialdehyde, GSH: Glutathione, CAT: Catalase

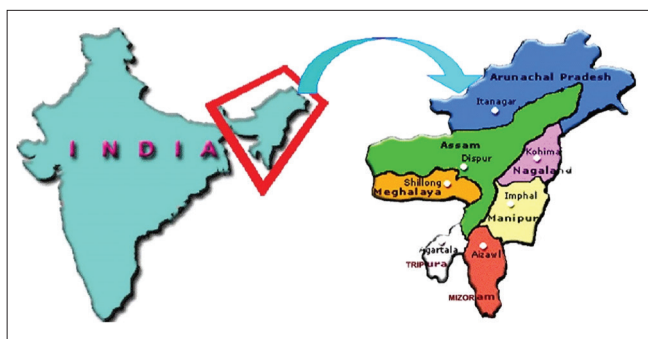


Figure 1: Geographical situation of North-East India

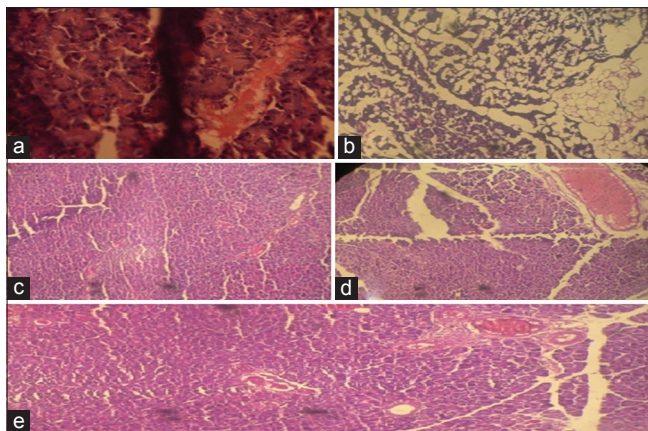


Figure 2: Effect of hydro-alcoholic stem bark extract on rat pancreas: (a) vehicle control, (b) diabetic control, (c) standard drug treated, (d) hydro-alcoholic stem bark extract 250 mg/kg body weight, (e) hydro-alcoholic stem bark extract 500 mg/kg body weight

and kidney have been observed during diabetes, and this may result in a number of deleterious effects due to the accumulation of O_2^- and H_2O_2 . CAT is a hem-containing ubiquities enzyme and also detoxify the H_2O_2 into water and oxygen. The level of CAT in liver and kidney was improved by HSBE and standard drug. Antioxidants may have a role in the prevention of diabetes.^[44] From the above *in vivo* antioxidant status, it is support to antidiabetic effect of this extract.

The histopathological studies of different group animals' pancreas were examined on the based on presence of secretory cells, the cell compactness, and degree of necrosis. The vehicle control slide shows the presence of cell compactness, and there is no cell integrity. The duct area is prominent and there is no fatty layer degeneration. The small scatter black spots supposed to be different pancreatic secretory cells. The pancreatic cell degradation is prominent in diabetic control (group II) slide. The cell integrity is totally loss. Significant fatty layer degradation has occurred. Small blackish, blood clotting spot is visible that may be sign of necrosis. The group III standard drug-treated cells' integrity is well managed. Although fatty layers degeneration has occurred. Small ballonic also seen but no blood clotting or necrosis has noticed. In the drug-treated 250 mg/kg (Group IV), fatty

layers degradation has noticed. The layers are stretches to longitudinally long area. The duct area is prominent and ballonic present. The compactness of cells is comparatively better than diabetic control. The cell regeneration has occurred through ductus portion. In case of Group V, slide pancreatic cell compactness has managed but fatty layer fragmentation has occurred frequently. Ballonic present, scattered blood clotting has notices but cell regeneration not progressively seen.

Insulin deficiency of β -cells was increased by immune cytochemical intensity. STZ-induced diabetic group reduced the number of immune-reactive insulin producing β -cells, and they were distributed in restricted pancreatic islets.^[22] On treatment of plant, extract modifies these abnormal changes. These histological studies were supported the present investigation. STZ was suspected to destroy pancreatic cells partially. The present investigation STZ-induced diabetic rats of the pancreas showed reduced islets cells and necrosis which were restored to near normal upon treatment with the extract. However, slightly more insulin producing β -cells in the standard drug metformin hydrochloride treated group was observed than in the diabetic group. Improved pancreatic exocrine activities can be described to insulin secretion from existing residual β -cells of islets or due to enhanced transport of blood glucose to peripheral. Histopathological report showed that the insulin producing β -cells are located generally in central region of pancreatic islets [Figure 2]. However, the cells are destroyed in STZ-inducing diabetes. Inhibition of these histomorphological changes and maintenance of normal architecture of insulin-producing cells by extract found to protect from the destruction of these cells by STZ.

CONCLUSION

In summary, findings from this study revealed that the hydro-alcoholic extract of the stem bark of *P. rubra* had effects to reduce blood glucose level to experimental rats. These also improved hyperlipidemia and other biochemical parameters. The extract had the activity to restore and regeneration of pancreatic β -cells upon treatment of diabetes (Type II). Further research on the molecular mechanism and the isolation of the compound responsible for these effects may lead to new bioactive compounds against Type II diabetes.

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