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Antidiabetic effect of aqueous extract of butea monosperma (LAM) Taub bark

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ABSTRACT

Herbal preparation of Butea monosperma (Lam.) Taub. bark had been considered as effective, economical and safe ethnomedicine for various ailments in Indian traditional system of medicine. The present study was aimed to investigate scientifically the antidiabetic potential of B. monosperma bark. Both kinds diabetes; insulin dependent diabetes mellitus (IDDM) and non insulin dependent diabetes mellitus (NIDDM) were induced in the rats by treating with alloxan monohydrate (150 mg/kg b wt ;ip) and hydrocortisone (5mg/100g b wt; ip) respectively. Fasting plasma glucose (FPG) levels were measured at periodic intervals during the test period. The blood samples were collected with care sino-ocular puncture method and serum was isolated by centrifugation to analyze plasma glucose and serum lipid profile. The results of preliminary phytochemical analysis depicts that B monosperma bark has the presence of steroids and tannins and absence of terpenoids, glycosides, alkaloids and flavonoids. The moisture content and total ash values of bark was 3.0%and 9.7% respectively. The treatment with bark aqueous extract of B monosperma substantially declined the plasma glucose level in both IDDM and NIDDM animal subjects by 7.2% and 26.6% respectively. This treatment also appreciably (P= 0.05 and P=0.01) lowered the serum lipid profile. In conclusion, the aqueous extract of Butea monosperma reflected hypoglycemic and hypocholesterolemic potential through glucose and lipid profile lowering activity in experimental animals. It supported the folklore state of antidiabetic potential of the plant.

Introduction

Diabetes is an intricate and multifarious group of disorders characterized by hyperglycemia that has reach epidemic extent in the present century [1]. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate [2]. Over the past 30 yrs, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe [3]. Several drugs such as biguanides and sulfonylureas are presently accessible to reduce hyperglycaemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome this problems[4].Management of diabetes without any side effects is still a challenge to the medical community. Plant kingdom represents a rich house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years[5, 6]. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in count with the body's own defense. Given a reasonable probability that medicinal plants with a long history of human use will eventually yield novel drug prototypes, systematic and intensive search in plants for new drugs to treat Type 2 diabetes mellitus seem to be of great utility [7]. Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. There is a well-known belief that green medicines are healthier and harmless or safer than synthetic ones. Earliest description of curative properties of medicinal plants was found in Rig Veda (2500-1800 BC). Charaka Samhita and Sushruta Samhita furnish extensive description on various medicinal herbs[8]. Medicinal plants such as Trigonella foenum graecum, Allium sativum, Gymnema slyvestre ,Mormordica charantia ,Zizyphus jujube ,Azadirachta indica and Syzigium cumini have been studied for treatment of diabetes mellitus[9,10]. As many of these plants were used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have side effects. Traditional antidiabetic plants might provide new oral hypoglycaemic compounds, which can counter the high cost and poor availability of the current medicines or drugs for many rural populations in developing countries[11].

In traditional medicine, there are many natural crude drugs that have the potential to treat many disease and disorders one of them is Butea monosperma (Lam.) Taub. (Palas), sacred tree, belongs to family Fabaceae and commonly known by various names viz., Flame of the Forest, Bastard Teak, Chichra tesu, Dhak and Palas etc. It grows throughout the Indian subcontinent; chiefly in Indo-Gangetic plains.Butea monosperma is extensibly used in Ayurveda, Unani and Homeopathic medicine. It is an erect, medium sized tree of 12-15 m high, with a crooked trunk, irregular branches and widely distributed in the country[12]. The plants of this genus are well known for their coloring

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matters and commonly used as tonic, astringent, aphrodisiac and diuretics[13]. Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumours). It is reported to possess antifertility, aphrodisiac and analgesic activities[14]. Flowers are free radical scavenging, antidiabetic, hepatoprotective and anti diarrheal[15]. The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite. The shoots are clothed with gray or brown silky pubescence. The bark is fibrous and ash coloured and reported to possess astringent bitter, pungent, alliterative, aphrodisiac and anthelmintic properties[16]. They possess a number of pharmacotherapeutic effects including antihepatotoxic, antifungal, estrogenic, anti- inflammatory, antistress and anticonceptive[17]. In view of its efficacy, free availability and having great potential of preventing various diseases, the present study looking into scientific exploration aqueous extract of butea monosperma bark as prospective antihyperglycemic and antihypercholesterolemic agents. Consequently, it is sensible to glimpse comprehensive studies on the efficiency, mechanism of action of plant extract on diabetic rats.

Materials and Methods

Plant material

The bark of Butea monosperma was collected from vicinity of Varanasi city. The plant was identified by experts of department of Dravyaguna, institute of Medical sciences, Banaras Hindu University (BHU), Varanasi, India. The bark was dried under the shade for 5-7 days in natural condition thereafter further processed at Ayurvedic pharmacy department, BHU for the preparation of aqueous extract.

Induction of diabetes in rats

Rats were made IDDM diabetic by single intraperitoneal injection of alloxan monohydrate (Koch light laboratories ltd; 150mg/kg body weight) for 3 consecutive days[18]. Alloxan was first weighted individually for each animal according to weight (solublized with 0.2ml saline) first prior to injection. Three days after alloxan injection, rats with plasma glucose levels of >200 mg/dl were included in the study. Like wise, rats were made NIDDM diabetic by administration of dissolved hydrocortisone sodium succinate (Glaxo Smith Kline, Pharmaceutical Ltd; 5 mg/100g b w, i.p. for eight consecutive days). NIDDM were confirmed in 48h after last cortisone dose administration. Only animals with glucose level >140mg/dl were used for the study.

Sample collection

Blood samples were collected with care by sino-ocular puncture method and serum was separated by centrifugation (5000 rpm for 10 min) under refrigerated conditions.

Experimental design

All the animals were randomly divided into the five groups with 8 rats in each group. The rats were used to study the effect of aqueous extracts of Butea monosperma bark (Bmb) on diabetes so induce. The rats were grouped and labeled as below

Group A – Normal control C1

Group B - Normal + Aqueous extract of Butea monosperma bark (Bmb)

Group C - Alloxan induced diabetic rats (Diabetic control C2; IDDM)

GroupD – Alloxan + Aqueous extract of Bmb

GroupE - Hydrocortisone induced diabetic rats (Diabetic control C3; NIDDM)

GroupF - Hydrocortisone + Aqueous extract of Bmb

Preparation of aqueous extracts of Butea monosperma bark (Bmb)

Water (deionized) and the coarse powder of the bark prepared at Ayurvedic Pharmacy Department, were mixed together in the ratio of 16:1 respectively. The mixture was further concentrated by evaporation of water by mild heat treatment till the water volume reduced to 1/8th of the original, and referred to as the decoction, was further concentrated by mild heating till it changed to semisolid form. Thereafter, it was dried in an oven at 60˚C for 24h. The resulting product (water soluble solid extract) is subsequently used as Bmb extract in the present manuscript hereafter. All the above procedures were performed in the department of Ras-Shastra, Institute Medical Science, BHU, Varanasi as per procedure given in treatise on Ayurveda (Sarngadhara Samhita, Slok 1-3)

Experimental Animals

Albino Wistar rats of both sexes having body weight 150-180g were obtained from central animal house of Institute of Medical Science, BHU Varanasi and were used in the experiment. Animals were kept in animal house at an ambient temperature of 25±2˚C and 50±5% relative humidity with a 14 h each of dark and light cycle. Animals were fed with pellet diet (Pashu Aahar Kendra, Varanasi) and distilled water. The study was approved by the Institutional Ethical Committee.

Treatment with the bark extract was started 48h after alloxan and hydrocortisone injections. Blood samples were taken at weekly intervals till the end of the study (i.e. 28th day) for estimating plasma glucose while serum profile parameters were measured on 0 and 28th day of treatment.

Preliminary Phytochemical Investigation of bark of Butea monosperma

The bark extract was subjected to qualitative tests for the identification of various active constituents viz Foreign matter (%w/w), extractive values (alcoholic and water soluble; % w/w); Moisture content (%), Ash values (Total ash and acid insoluble ash; %w/w) and chemicals (Alkaloids, Terpenoids, Steroids, Tannins, Saponins, Glycosides and flavonoids) using standard test procedures[19, 20].

Biochemical Analysis

Biochemical parameters plasma glucose estimated by GOD-POD method[21], Serum total cholesterol (S. TCh) estimated by CHOD/PAP method[22]. Serum Triglyceride (S. TG) carried out by enzymatic method[23] and Serum high density lipoprotein – cholesterol (S. HDL-C)[24] and Serum low density lipoprotein – cholesterol (S LDL-C) and very low density lipoprotein-cholesterol (S VLDL-C)[25] calculated as per equation:

VLDL–C = Serum TG/5

LDL–C = Serum T Ch-(Serum VLDL–C + Serum HDL–C)

Serum albumin was estimated by BCG method[26] and Serum urea was carried out by enzymatic method[27.]

Statistical Analysis

All the values of plasma glucose level and other biochemical estimations were expressed as mean±SD are analysed for student‘t’ test differences between the groups were considered significant at $P \leq 0.05$ & $P \leq 0.01$ levels.

Abbreviations

AllXN, Alloxan; DM, Diabetes Mellitus; Bmb, Butea monosperma Bark; FPG, Fasting plasma glucose, TCh, Total cholesterol; TG, Triglyceride, HDL–C, High density lipoprotein-Cholesterol; LDL–C, Low density lipoprotein-Cholesterol, IDDM, Insulin dependent diabetes mellitus; NIDDM, Non insulin dependent diabetes mellitus; GOD–POD, Glucose oxidase peroxidase ; CHOD/PAP, Cholesterol hydrolysis and oxidation.

Results

Preliminary phytochemicals screening

The phytochemical parameters like foreign matter (2.0%), Extractive alcohol and water soluble values (15.5 and 16.0%), moisture content (3.0%), total ash (9.70%) and acid insoluble ash (1.3%) of bark was investigated. Prelude phytochemicals screening of bark showed the existence of steroids, tannins and glycosides, terpenoids, alkaloids and flavonoids were lacking in bark of Butea monosperma as depicted in table1.

Effect of aqueous extract Bmb on normoglycemic rats

Table 2 reveals the effect of aqueous extract of Bmb on normal rats during the course of 28 days of treatment. It was found that the onset of values was 82.98±9.8 mg/dl and after 28 days treatment the fasting plasma glucose was significantly reduced to 80.28±9.8 mg/dl i.e. 6.3% reduction was seen. This study also support that 1.25 g/kg b wt/d is effective dose to reduce FPG level of animal subjects.

Effect on fasting Plasma glucose (FPG) level on diabetic rats

In all groups, prior to alloxan and hydrocortisone administration, the basal blood glucose levels of rats were not significantly different, However after alloxan and hydrocortisone blood glucose level were significantly higher i.e. above >200 mg/dl for IDDM and >140 mg/dl for NIDDM group and these animals were selected for the study. The non diabetic control (group A) and treated (group B) with aqueous extract of Bmb remained constantly euglycemic through the course of the study.

Table 2 depicts the effect of aqueous extract of Butea monosperma bark on fasting plasma glucose level of both alloxan & hydrocortisone induced diabetic rats. In test animals suffering from IDDM (alloxan; group D), the fasting plasma glucose level was 241.80±7.70mg/dl for group D, which substantially declined to 224.20±5.21 mg/dl after 28 days of intervention. Hence, 7.2% reduction was noticed due to treatment of aqueous extract of Bmb in a dose of 1.25 g kg^{-1} b wt. Similarly, NIDDM subjects (group F) were also significantly reduced as marked from144.6±18.90 to 106.01±22.01 mg/dl after 28 days of intervention i.e 26.6%, notably reduction was seen in comparison to group E (diabetic control C3 for NIDDM).

The data has been pinpointing of the momentous effect of intervention at $P \leq 0.01$ level when the values were compared to the onset values. In other evaluation at the end (28^{th} day) of the study, the final values of both experimental groups (D&F) were compared with that of placebo groups (C&E) and again it went to point towards a significant difference at $P \leq 0.01$.

Effect on Serum lipid profile

The Serum lipid profile of aqueous extract of Bmb fed and control animals are presented in Table 3. After 28 days Intervention, both experimental groups (Group D; alloxan treated IDDM & Group F; hydrocortisone treated NIDDM) treated with Bmb showed significant decrease in serum lipid profile $P \leq 0.05$ level. The data shows that alloxan induced rats treated with aqueous extract of Bmb had significant reduction in T-Ch 225.80±14.0 mg/dl to 186.70±7.20 mg/dl and in hydrocortisone induced rats, 174.80±14.0 mg/dl to 117.0±15.8 mg/dl at 21 days respectively. Similar results were seen with LDL-C (129.04±6.2 mg/dl) and VLDL-C (59.96±10.8 mg/dl) also when compared with diabetic control (2164.78±9.8 mg/dl) and C3 (113.76±9.80 mg/dl). No significant reduction was observed in serum TG levels of all experiment groups. The data also found that the serum albumin and urea were significantly raised in both treated group in the present study. However the rise was insignificant (Table-3).

Discussion

Diabetes mellitus (DM) is a major health problem worldwide in recent time and Asia and Africa are the most viable areas where the disease is feared to raise 2–3 folds. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues[28]. The important values of some plants and their parts have long been published but a large number of them remain unexplored as yet. So there is a need to investigate their uses and to accomplish pharmacognostic and pharmacological studies to make certain their therapeutic properties. There are many hypoglycemic plants known through the folklore but their introduction into the modern therapy system awaits the discovery of animal test system that closely parallel to the pathological course of diabetes in human being.

The present study, the antidiabetic potential of aqueous extract of Butea monosperma barks were evaluated in both Alloxan and hydrocorticone diabetic rats. Alloxan and hydrocorticone causes diabetes through its ability to destroy the insulin producing beta cells of pancreas[29]. In vitro studies have beta cells causing cell necrosis[30]. The cytosolication of alloxan is mediated by reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration, leading to rapid destruction of beta cells[31]. Likewise hydrocortisone succinate has also potential mechanism for the inhibition of insulin release following an acute dose of hydrocortisone sodium succinate (300mg/kg intraperitoneally) elevate plasma glucose level when administered to male swiss wester without altering plasma insulin levels[32].

The results indicate that the extract of Butea monosperma bark decreases the serum glucose in normal rats as compared to the normal control C1 groups.The maximum hypoglycemic activity of extract was observed in NIDDM animal models that is 26% This is might be due to increasd peripheral glucose utilization or potentiating of insulin effect. Stigmasterol, is sterol isolated from the bark of Butea monosperma (2.6 mg/kg/d for 20 days) was evaluated for thyroid hormone and glucose regulatory efficacy in mice. The result showed its thyroid inhibiting and hypoglycemic properties. Antioxidative potential due to decrease in the hepatic lipid peroxidation and an increase in the activities of catalase , superoxide dismutase and glutathione [33] . Similar study indicated by[34] that the single dose treatment of ethanolic extract of Butea monosperma flowers at the dose of 200mg/kg P.O. significantly improved glucose tolerance and cause reduction in blood glucose level in alloxan induced diabetic Rats. Oral administration of the ethanolic extract of the Butea monosperma seeds at the dose of 300mg/kg b.w., exhibited significant antidiabetic, hypolipidaemic and antiperoxidative effects[35.] A similar kind of study conducted in the bark of Ficus hispida has shown a significant blood glucose reducing effect in normal and diabetic rats[36] .The oral administration of bark extract at 1.25mg kg-1 showed the significant decrease in the fasting glucose level at the end of study after 28 days. According to study conducted by[37], treatment of diabetes mice with ethanolic extract of Butea monosperma (300mg/kg body wt) for 45 days caused significant reduction in fasting blood glucose level.

The elevated T-Ch, TG, LDL-C and VLDL-C and decreased HDL-C level in both alloxan and hydrocortisone induced diabetic rats were in agreement with previous reports regarding alteration of these parameters under diabetics induced

hyperlipidemia might be due to excess mobilization of fat from the adipose tissue because of underutilization of glucose[38] . The mean serum T-Ch of the experimental groups had been significantly reduced after administration of Bmb aqueous extract when compared with placebo groups. TG and VLDL- C which influence lipid deposition are clotting mechanism have been reduced significantly in hydrocortisone diabetic rats through the reduction was not sizable in alloxan diabetic rats. As the Bmb aqueous extract have been found to be positive modulator of lipid profile of diabetic subjects in being hypocholesterolemic with respect to T Ch,TG, LDL-C and VLDL-C level in serum, they also have static effect on HDL-C which can build synergy of their effects. HDL-C appears to remove cholesterol from the walls of arteries and returns it to the liver and reduces the risk of heart attack[39]. Thus, they can improve the lipid profile along with serum urea and serum albumin. In study conducted by [40], has been observed that administration of Azadirachta indica seed kernel powder significantly decreased the concentration of serum lipids are blood glucose in alloxan diabetic rats. In another study concluded by[41] that leaf extract of Aegle marmelos and aqueous extract of Terminalia arjuna were reported to act as hypocholesterolemic[42]. The efficacy of the bark extract of the test plant in lessening diabetes mainly depends on the presence of certain active principles. However, few active principles of Butea monosperma are already known. From the study, we can conclude that aqueous extract of Butea monosperma bark has beneficial effect on blood glucose level. It has the credible to report therapeutic effect in diabetes. Further pharmacological and biochemical studies are required to elucidate the mechanism action of the extracts in details at molecular level and also need to investigate the antioxidant potential are free radicals.

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Table-1 Preliminary Phytochemical Analysis of Butea monosperma (Lam.)Taub Bark

Phytochemicals	Butea monosperma
Foreign matter(%w/w)	2.0%
Extractive value(%w/v)	
Alcohol soluble	15.5%
Water soluble	16.0%
Moisture content	3.0%
Ash values	
Total Ash (%w/w)	9.7%
Ash insoluble ash (%w/w)	1.3%
Chemical Tests	
Alkaloids	-ve
Terpenoids	-ve
Steroids	+ve
Saponins	-ve
Tannins	+ve
Glycosides	-ve
Flavonoids	-ve

Table-2.Effect of daily oral dose of aqueous extract of Butea momosperma bark (Bmb) on serum glucose of alloxan & hydrocortisone rats

Group	Treatments	Mean Serum glucose level mg/dl						
		Before Treatment		After Treatment				%Decrease
		BT	AT	AT_1	AT_2	AT_3	AT_4	
A	Normal C1 (Control)	80.98±10.8	82.98±9.80	81.21±11.20	80.60±9.91	0.98±10.8	80.28±9.80	6.3%
B	Normal+Aqueous Extract Bmb	85.35±6.24	-	85.38±8.43	83.24±9.8	81.43±8.9	79.90±7.54^{ae}	
C	Diabetic Control C2 (Alloxan)	66.91±7.5	237.12±10.1	243.21±9.31	246±10.8	248.51±10.5	252.80±10.21	7.2%
D	Alloxan+Aqueous Extract Bmb	66.13±3.81	241.80±7.70	233.31±5.50	230.10±5.41	226.50±5.01	224.20±5.21^{be}	
E	Diabetic Control C3 (HYD CORT)	71.50±4.30	158.70±17.21	163.40±15.91	165.90±16.60	168.80±17.81	166.20±16.01	26.6%
F	HYD CORT Aqueous Extract Bmb	75.37±10.9	144.61±18.9	128.48±22.1	116.40±23.81	110.80±22.6	106.01±22.01^{be}	

Value: mean±SD (n=8)
ap ≤ 0.05, bp≤ 0.01, cp≥ 0.05 when AT values compared with AT4 values of respective groups .
dp≤0.05, ep≤0.01, fp≥0.05 when AT4 values of control (C2 & C3) compared with experimental groups (D & F)
Group A- Normal control (C1)
Group B- normal treated + Aqueous extract of Bmb.
Groups (C&E)-Diabetic control (C2&C3)
Groups (D&F) given test sample (Aqueous extract of Bmb)
BT- Before treatment, AT- After Alloxan/ Hydrocortisone
AT_1, AT_2, AT_3, AT_4 are alloxan/ Hydrocortisone treated after 7, 14, 21 and 2 days intervention.

Table -3. Effect of aqueous extract of Butea monosperma Bark (Bmb) on serum lipid profile (150mg/kg bw) and hydrocortisone (5 mg/100g bw) induced diabetic rats after 28 days of treatment.

Group No.	Treatments	Mean Serum glucose level mg/dl						
		Serum TCh	Serum TG	Serum HDL-C	Serum LDL-C	Serum VLDL-C	Serum Albumin	Serum Urea
A	Normal Control C1	98.6±4.80	106.31±10.70	34.6±4.20	44.90±5.4	23.27±2.01	2.86±0.24	34.45±2.25
B	Normal Control +Aqueous Extract Bmb	86.78±8.0[a]	107.45±8.9[c]	35.8±5.2[a]	42.9±4.8[a]	21.72±0.92[a]	3.7±1.48[a]	35.82±3.20[a]
C	Diabetic Control C2 (ALLXN)	225.80±14.0	107.10±7.8	39.60±3.43	164.78±9.8	21.42±1.61	2.21±0.34	37.81±2.80
D	ALLXN+Aqueous Extract Bmb	186.70±7.20[b]	106.80±8.21[c]	36.30±1.71[a]	129.04±6.2[b]	21.36±1,91[c]	2.31±0.24[b]	36.28±2.81[c]
E	Diabetic Control C3 (HYD CORT)	174.80±14.0	126.20±15.2	35.80±3.41	113.76±9.80	25.4±2.41	2.41±0.25	35.82±3.81
F	HYD CORT+ Aqueous Extract Bmb	117.0±15.8[b]	106.20±15.21[b]	32.83±2.82[a]	59.96±10.8[b]	21.21±2.82[a]	5.88±0.26[b]	34.92±2.62[c]

Value: mean±SD (n=8)

ap≤0.05, bp≤0.01, cp≥0.05 when AT4 value of control groups A, C&E (normal alloxan and hydrocortisone induced diabetic rats) compared with experimental groups (B,D& F).

Group A- Normal control (C1)

GroupB- Normal + Aqueous extract of Bmb.

Groups (C&E)-Diabetic control (C2&C3)

Groups (D&F) given test sample (Aqueous extract of Bmb)