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ANTIDIABETIC ACTIVITY ETHANOLIC LEAVES EXTRACT OF *BAUHINIA GALPINII* LINN. ALLOXAN INDUCED DIABETIC IN RATS

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Keywords:

Bauhinia galpinii Linn. Alloxan, Antidiabetic activity, Blood glucose level, Glucose Tolerance test, Antihyperglycemic effect

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ABSTRACT: Aim of the study: This study aimed to investigate the antidiabetic activity of ethanolic leaves extracts of *Bauhinia galpinii* Linn. alloxan, induced diabetic in rats. **Materials & methods:** The adult male albino rats of weight 180-240 gm were selected for the study. The Dose of 100 mg/kg, 250 mg/kg and 500 mg/kg of ethanol extract was selected for the test. All the doses were given orally after making an emulsion in the vehicle, *i.e.*, 1% acacia gum and the standard drug, i.e., glimepiride was given orally (10 mg/kg) in the vehicle. Diabetes mellitus was induced by administering an intraperitoneal injection of alloxan monohydrate 120 mg/kg to the overnight fasted rats. A blood sample was collected from tail nipping, and an automatic electronic glucometer determined glucose level. Ethanolic extract of *Bauhinia galpinii* Linn. leaves were subjected to anti-diabetic activity in rats where alloxan monohydrate (120 mg/kg b.w., i.p.) used as the diabetogenic agent. A marked rise in fasting blood glucose level observed in diabetic control compared to normal control rats. **Results:** In the present Ethanolic extract of *Bauhinia Galpinii* Linn (at 250 and 500 mg/kg) exhibited a dose-dependent significant anti-hyperglycemic activity on 4th, 7^{th,} and 10th-day post-treatment. The extract dose of 100 mg/kg also caused a reduction in blood glucose level, but the results were found statistically insignificant. The antihyperglycemic effect of ethanol extract was found less effective than the reference standard. Glibenclamide produced a significant reduction in blood glucose compared to diabetic control. When the glucose tolerance test did the activity of the extract in glucose-loaded rats, ethanolic extract showed a significant effect on the blood glucose level, but an extract of 100mg/kg did not show the significant result. Ethanolic extract 250mg/kg and 500mg/kg showed a significant decrease in blood glucose level. **Conclusions:** The ethanolic extract of Bauhinia galpinii Linn. has a protective effect against alloxan, induced diabetic in rats.

INTRODUCTION: Five thousand years ago in the magnificent Himalayas, one of the greatest sages of India, Srila Vyasadeva wrote down the Vedas for the first time; this included a branch which is called Ayurveda: "The science of Life" (Ayur means life and Veda means science).

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The Vedas came from an oral tradition that reached back into antiquity. Srila Vyasadev entrusted the original copies of the texts with his most erudite and enlightened disciples, who, along with other great sages, inaugurated a very long sacrificial ceremony for hundreds of years for the purification and blessings of the entire world.

Remember people lived for one to two thousand years back then. During that time, they studied and discussed these ancient texts with their disciples, who wrote commentaries, and expanded and developed these original and eternal truths without ever altering them [1].

There is information about atomic energy, gynecology, pediatrics, surgery, anatomy, herbal drugs, Ayurvedic dieting, and nutrition. All are described most simply and profoundly to make it easy enough for any person to have a basic working knowledge of this great science of life - Ayurveda. I know this sounds incredible and you may be wondering, "How is it possible to have one system embrace all systems" How would it be applied? The answer is simple. The first step is to ascertain the individual's "Biological Mode," and then to treat the person accordingly [2].

Generally speaking, most people are a combination of two modes. One is the primary, and the other is secondary. But there are those who are purely dominated by one mode, and in rare cases, those who are a mixture of all three. This elemental theory broken down into divisions of modes identifies not only body types for humans, but also for animals, vegetables, plants, herbs, geographical locations, times of day, seasons of the years, and activities performed [3].

Diabetes mellitus is a common disease in the United States. It is estimated that over 16 million Americans are already caught with diabetes, and 5.4 million diabetics are not aware of the existing disease. Diabetes prevalence has increased steadily in the last half of this century and will continue rising among the U.S. population. It is believed to be one of the main criterions for deaths in the United States, every year. This diabetes information hub projects on the necessary steps and precautions to control and eradicate diabetes, completely.

Diabetes is a metabolic disorder wherein the human body does not produce or properly uses insulin, a hormone that is required to convert sugar, starches, and other food into energy. Diabetes mellitus is characterized by constant high levels of blood glucose (sugar). The human body has to maintain the blood glucose level at a very narrow range, which is done with insulin and glucagon. The function of glucagon is causing the liver to release glucose from its cells into the blood, for the production of energy [4].

There are three main types of diabetes:

- Type 1 diabetes
- Type 2 diabetes
- Gestational diabetes

Type 1 and Type 2 diabetes impede a person's carefree life. When the breakdown of glucose is stopped completely, the body uses fat and protein for producing energy. Due to this mechanism symptoms like polydipsia, polyuria, polyphagia, and excessive weight loss can be observed in a person with diabetes. The desired blood sugar of human body should be between 70 mg/dl -110 mg/dl at fasting state. If blood sugar is less than 70 mg/dl, it is termed as hypoglycemia and if more than 110 mg /dl, it's hyperglycemia.

Diabetes is the primary reason for adult blindness, end-stage renal disease (ESRD), gangrene and amputations. Overweight, lack of exercise, family history and stress increase the likelihood of diabetes. When the blood sugar level is constantly high, it leads to kidney failure, cardiovascular problems, and neuropathy. Patients with diabetes are 4 times more likely to have coronary heart disease and stroke. Also, Gestational diabetes is more dangerous for pregnant women and their fetus.

FIG. 1: *BAUHINIA GALPINII* LINN.

The present investigation was therefore taken up to establish an identity of fresh and dried leaves antidiabetic activity for the standardization of the drug. *Bauhinia galpinii* Linn. is a species of shrub in the family Fabaceae. It is endemic to parts of eastern and southern Africa, where its popular name is "Pride of De Kaap." In other places,

however, it is variously known as Orchid Tree, Red Bauhinia, Nasturtium Bauhinia, African Plume, Red Orchid Bush, and by other informal names. The species name commemorates E. E. Galpin.

Bauhinia is a genus of more than 200 species. The genus was named after the Bauhin brothers, Swiss-French botanists. The leaves share the double-leaf configuration of a heart, or more popularly, that of a butterfly.

Scientific Classification:

Kingdom : Plantae
(Unranked) : Angiosperms
(Unranked) : Eudicots
(Unranked) : Rosids
Family : Fabaceae
Genus : Bauhinia
Species : *Bauhinia galpinii* Linn.

Synonyms: *Perlebia galpinii* (N.E.Br.) A. Schmitz, Bauhinia punctatasensu Bolle [5].

Pharmacological Activity:

Anti-Inflammatory/Antipyretic/Antinociceptive: Study of aqueous extract on animal models showed antinociceptive, antipyretic and anti-inflammatory activities [6].

Nephroprotective: The study showed the unripe pods, and ethanol extract of leaves of BP significantly protected rat kidneys from gentamicin-induced histopathological changes, with normalization of gentamicin-induced increases in serum creatinine, uric acid and BUN [7].

Wound Healing: Study of methanol and chloroform extracts prepared in carpool and simple ointment base and applied to wounds showed a dose-dependent and significant reduction in epithelization and wound contraction time. The methanol extract exhibited more activity. Results indicated BP leaves had wound healing property [8].

Antiproliferative / Antioxidant: The study showed *Bauhinia purpurea* leaf possesses potential antiproliferative and concentration-dependent antioxidant activities.

MATERIALS AND METHODS:

Collection of Plant: The fruits of *Bauhinia Galpinii* Linn. (Family: Fabaceae) were collected during August 2015 from Jhansi District, (U.P.), India.

Collection and Authentification:

Identification and Authentication: The identification and authentication of the plant were done by Director, Indian Grassland and Fodder Research Institute, Gwalior Road, Jhansi, Uttar Pradesh, India. A voucher specimen of the plant was kept in the herbarium of Indian Grassland, and Fodder Research Institute, Jhansi with the accession number of the example is on date 25/01/2016. SKS/GSM 2016,

Preparation of Extraction: The air-dried in shade plant leaves of *Bauhinia galpinii* (250gm) was coarsely powdered using a grinder and continuously extracted in a Soxhlet apparatus at 50ºC with 750 ml of Petroleum ether than 750 ml of ethanol. The extract was filtered through a paper filter (Whatman, no. 1) and evaporated under reduced pressure by the rotary evaporator.

Extractive Value: Extracts were prepared with various solvents. Percentages of the extractive values were calculated concerning the air-dried drug are given in **Table 1.**

TABLE 1: EXTRACTIVE VALUE OF *BAUHINIA GALPINII* LINN.

S. no.	Types of solvents	% w/w
1	Petroleum ether (60-80 ºC)	2.248
2	Ethanol 95%	18.384

Extraction of Plant Materials: 250 g coarse powder of air-dried fruits of *Coccinia indica* were packed in a muslin cloth and subjected to Soxhlet extractor for continuous hot extraction with petroleum ether and ethanol for 8 hrs separately. Then each extract was filtered, and the filtrate was evaporated to dryness.

Preliminary Phytochemical Studies: Phytochemical tests were done in plant extracts for the detection of the presence of different chemical constituents such as; alkaloids, glycosides, steroids, flavonoids, essential oils, carbohydrates, proteins, tannins and other substances which are responsible for the biological activity. So, the chemical tests are performed in the Ethanolic extract (EE) of *Bauhinia Galpinii* Linn. for the detection of different chemical constituents:

Chemical (Drugs & Solutions) and Apparatus: Alloxan of CDH, New Delhi was used for the inducted of diabetes and was obtained from the Department of Pharmacy, and the standard drug, *i.e.* glimepiride was received by Ross Robinz Biotech, Solan (H.P).

Preparation of Dose: The Dose of 100 mg/kg, 250 mg/kg and 500 mg/kg of ethanol extract was selected for the test. All the doses were given orally after making an emulsion in vehicle, *i.e.* 1% acacia gum and the standard drug, *i.e.* glimepiride was given orally (10 mg/kg) in the vehicle.

Animal Selection:

Animals: The adult male albino rats of weight 180-240 gm were selected for the study. All animals were procured from disease-free animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. The Institute of Pharmacy is approved by the Institutional Animal Ethical Committee (BU/ Pharm/IAEC/13/12). The animals were housed in polypropylene cages, 5 per cage with free access to standard laboratory diet and water ad *libitum*. The rats were maintained under standard laboratory conditions at 25±2^{0}C relative humidity 50±15% and normal photoperiod (12 h dark/ 12h light) were used for the experiment.

Animal Groups: After checking the fasting blood glucose in overnight fasted diabetic rats. They were divided into five groups of five rats reach and one group of non-diabetic rats.

All the doses were given in the following manner

- Group A served as normal control and did not receive any treatment.
- Group B served as diabetic control and received alloxan monohydrate and vehicle (0.2 ml of 2% aqueous gum acacia)
- Group C alloxan monohydrate + Glimepiride (10 mg/kg p.o) and served as the standard
- Group D alloxan monohydrate +ethanolic extract (100 mg/kg p.o)
- Group E alloxan monohydrate + ethanolic extract (250 mg / kg p.o)
- Group F alloxan monohydrate + ethanolic extract (500 mg / kg p.o) [9]

The treatment was continued for 3 h. During this period, food and water were supplied ad *libitum*. All the doses were administered orally by the oral feeding needle. The effect of the extract on blood glucose levels was estimated on overnight fasted rats on the hour 0, 1, 2, and 3 by the method described before. The basal values are those of the day on which extract was started to give. The general behaviors of the animals were recorded daily. The blood glucose level in (Mean ± S.E.M.) is shown in the Table.

Design of Work: In this glucose tolerance test fasted five groups of five animals each group 1 served as control and received the vehicle. Group 2 received standard drug glimepiride at on i.p dose of 120 mg/kg and group 3,4 and 5 received ethanolic extract orally at a dose of 100 mg/kg, 250 mg/kg and 500 mg/kg. The rats of all the group were given glucose (2g/kg), 30 min after the extract and drug administration. Blood samples were collected by tail nipping just before glucose administration 0 h, 30 min, 60 min, and 120 min after glucose loading and blood glucose level were measured by glucometer. Basal value is those after which glucose was administrated.

Effect of Ethanolic Extract on Oral Glucose Tolerance Test: The hypoglycemic effect of ethanolic extract of *Bauhinia Galpinii* Linn. studied on glucose-loaded rats.

Protocol: In this glucose tolerance test fasted five groups of five animals each group 1 served as control and received the vehicle. Group 2 received standard drug glimepiride at on i.p dose of 120 mg/kg and group 3, 4 and 5 received ethanolic extract orally at a dose of 100 mg/kg, 250 mg/kg and 500 mg/kg. The rats of all the group were given glucose (2g/kg), 30 min after the extract and drug administration.

Blood samples were collected by tail nipping just before glucose administration 0 h, 30 min, 60 min, and 120 min after glucose loading and blood glucose level were measured by glucometer. Basal value is those after which glucose was administrated.

Assessment of Antidiabetic Activity:

TABLE 2: EFFECT OF *BAUHINIA GALPINII* LINN. LEAVES ON FASTING BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC RATS ALLOXAN 120 mg/kg i.p

Group	Treatment	Fasting blood glucose level (mg/dl)			
		Basal value	4th day	7th day	10th day
A	Normal control	90.46 ± 3.80	92.82±2.92	92.32±1.73	88.29±3.44
B	Diabetic control (Vehicle)	293.8±5.27	286.91±5.05	291.8±5.41	289.41±9.75
C	Alloxan + glibenclamide (10 mg/kg)	285.86±6.92	205.25±7.06***	183.18±6.35***	178.13±6.20***
D	Alloxan + Ethanolic extract (100 mg/kg)	291.76±4.79	277.76±5.65	266.23±8.19	255.42±7.71
E	Alloxan + Ethanolic extract (250 mg/kg)	284.48±5.32	258.23±6.66*	255.85±9.97**	252.06±9.19**
F	Alloxan + Ethanolic extract (500mg/kg)	287.48±5.32	212.61±5.07***	198.36±3.52***	189.83±3.31***

Value are mean + SEM, n=6, *P<0.05, **P<0.01 and ***P<0.01 *vs*. diabetic control

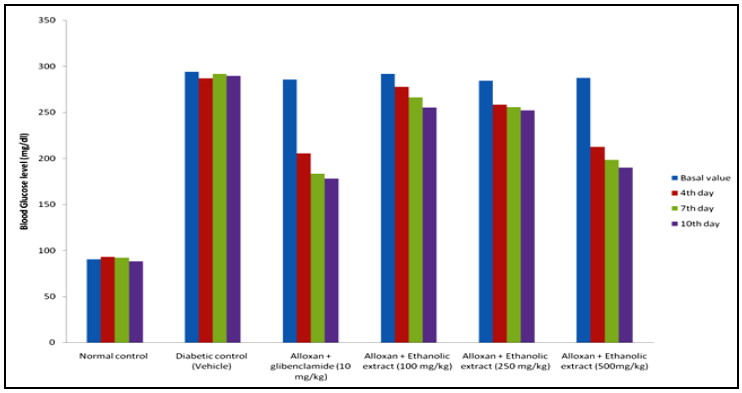

FIG. 2: THE ANTIHYPERGLYCEMIC EFFECT OF ETHANOLIC EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

TABLE 3: THE ANTIHYPERGLYCEMIC EFFECT OF ETHANOLIC EXTRACT ON GLUCOSE LOADED RATS

Group	Dose mg/kg	Blood glucose level (mg/dl)				% lowering of BGL compare to the basal value
		0 hours	30 min	60 min	120 min	
I	Control (2gm /kg)	78.2±3.20	168.8±1.35	143.8±2.37	130.3±1.84	……..
II	Glibenclamide (120 mg/kg)	76.5±2.45	145.2±3.35**	111.5±3.11**	82.4±2.40**	43.44%
III	Ethanolic extract (100 mg/kg)	72.2±2.37	152.2±1.77**	136.2±1.76 (NS)	124.2±2.26 (NS)	18.42%
IV	Ethanolic extract (250 mg/kg)	73.3±3.23	157.3±2.56**	130.2±3.26	114.8±3.05	27.38%
V	Ethanolic extract (500 mg/kg)	79.3±3.30	150.8±264**	122.4±2.40**	90.5±1.70**	40.00%

NS= not significant, **P< 0.01, show significant when compare with control

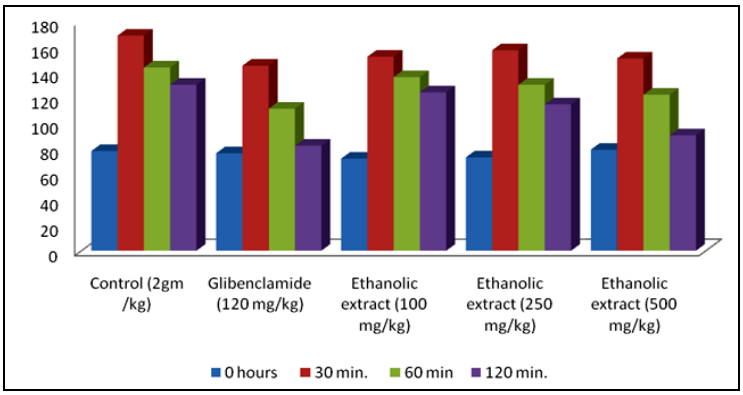

FIG. 3: THE ANTIHYPERGLYCEMIC EFFECT OF ETHANOLIC EXTRACT ON GLUCOSE LOADED RATS

Statistical Analysis: The data were statistically evaluated using one-way ANOVA. Expressed as Mean ± S.E.M. followed by Tukey test using the Graph pad instant Demo (Data set 1.IS) version P. values of 0.05 or less were considered to be significant.

RESULTS: Ethanolic extract of *Bauhinia Galpinii* Linn. leaves were subjected to anti-diabetic activity in rats where alloxan monohydrate (120 mg/kg b.w., i.p.) used as the diabetogenic agent. A marked rise in fasting blood glucose level observed in diabetic control compared to normal control rats. Ethanolic extract of *Bauhinia Galpinii* Linn (at 250 and 500 mg/kg) exhibited a dose-dependent significant anti-hyperglycemic activity on 4^{th}, 7^{th}, and 10^{th} day post-treatment. The extract dose of 100 mg/kg also caused a reduction in blood glucose level, but the results were found statistically insignificant. The antihyperglycemic effect of ethanol extract was found less effective than the reference standard. Glibenclamide produced a significant reduction in blood glucose compared to diabetic control.

When the activity of the extract was done by the glucose tolerance test in glucose-loaded rats, ethanolic extract showed a significant effect on the blood glucose level, but an extract of 100 mg/kg did not show the significant result. Ethanolic extract 250 mg/kg and 500 mg/kg showed a significant decrease in blood glucose level.

The ethanolic plant extract was subjected for the phytochemical screening for the detection of various plants constituents, and it's found that flavonoids, alkaloids, and phenolic compounds are presented as major active principle.

The best solvent system for TLC of *Bauhinia galpinii* Linn. is Toluene: Ethyl acetate: few drops of Acetic acid (7:3 few drops). TLC of *Bauhinia galpinii* Linn. shows the presence of seven compounds with different R_f values in Different color using the 0.5% Vanillin in Dil. H_2SO_4 detecting reagent which suggests that the presence of seven compounds in the extract.

HPTLC was carried out by PG Tech Pvt. Ltd., Indore Madhya Pradesh India, using solvent system Toluene: Ethyl acetate in the ratio of 70:30 and HPTLC of extract show the six peaks confirming that the six compounds may be present in the ethanolic extract of the leaves of *Bauhinia galpinii* Linn.

Ethanolic extract of *Bauhinia galpinii* Linn. leaves were subjected to anti-diabetic activity in rats where alloxan monohydrate (120 mg/kg b.w., i.p.) used as the diabetogenic agent. A marked rise in fasting blood glucose level observed in diabetic control compared to normal control rats. Ethanolic extract of *Bauhinia galpinii* Linn (at 250 and 500 mg/kg) exhibited a dose-dependent significant anti-hyperglycemic activity on 4^{th}, 7^{th}, and 10^{th} day post-treatment. The extract dose of 100 mg/kg also caused a reduction in blood glucose level, but the results were found statistically insignificant. The antihyperglycemic effect of ethanol extract was found less effective than the reference standard. Glibenclamide produced a significant reduction in blood glucose compared to diabetic control.

When the activity of the extract was done by the glucose tolerance test in glucose-loaded rats, ethanolic extract showed a significant effect on the blood glucose level, but an extract of 100mg/kg did not show the significant result. Ethanolic extract 250mg/kg and 500mg/kg showed a significant decrease in blood glucose level.

CONCLUSION: It can be concluded that the present study on *Bauhinia Galpinii* Linn (at 250 and 500 mg/kg) exhibited a dose-dependent significant anti-hyperglycemic activity on 4th, 7th, and 10th-day post-treatment. The extract dose of 100 mg/kg also caused a reduction in blood glucose level, but the results were found statistically insignificant. The antihyperglycemic effect of ethanol extract was found less effective than the reference standard. Glibenclamide produced a significant reduction in blood glucose compared to diabetic control. When the activity of the extract was done by the glucose tolerance test in glucose-loaded rats, ethanolic extract showed a significant effect on the blood glucose level, but an extract of 100mg/kg did not show the significant result. Ethanolic extract 250mg/kg and 500mg/kg showed a significant decrease in blood glucose level. Leaves of *Bauhinia Galpinii* Linn. better antidiabetic activity but it shows mild to moderate antidiabetic activity against alloxan, induced diabetic in rats.

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CONFLICT OF INTEREST STATEMENT: We declare that we have no conflict of interest.

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