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RESEARCH ARTICLE

"EXPERIMENTAL STUDY OF ATIBALA (ABUTILON INDICUM LINN SWEET) ROOT WITH EQUAL QUANTITY OF SUGAR (SITA) AND COW MILK (GODUGDDHA) IN RELATION TO ANTI-DIABETIC ACTIVITY IN STREPTOZOTOCIN INDUCED ALBINO WISTAR RATS"

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Abstract

Background: Diabetes mellitus, a clinical syndrome developed due to absolute or relative deficiency of insulin and it is mainly characterized by hyperglycemia. It has high prevalence and it is fast gaining status of potential epidemic. That's why many synthetic drugs are available to treat the diabetes which is costly and giving serious side effects after long use. The herbs are safe and cost effective to treat diabetes. Ayurved classic (Bhavapraksh Nighantu) mentioned Atibala root with Sita (sugar) and Godugddha (Cow milk) have anti-diabetetic activity. As the Diabetes mellitus is most considerable disease by ethical issues hence it is decided to evaluate anti-diabetic activity of Atibala with sugar and cow milk in streptozotocin induced Albino Wistar rats.

Aim and Objectives: To study Atibala root with equal quantity of sugar and cow milk as anti-diabetic activity.

Methodology: In this study, 30 Albino Wistar rats will be divided randomly into 5 groups (6 in each). Test drugs will be administered for 21 days. The random blood glucose will be measured 0, 7, 14 & 21th days of drug administered. Anti-diabetic activity will be evaluated on biochemical parameter 0 day & at 21thday and on 22th day histopathological study of pancreas.

Results: Changes will be observed in the objective outcomes**.**

Conclusion: Atibala root with equal quantity of sugar and cow milk will have anti-diabetic activity.

Introduction:-

Diabetes mellitus, a clinical syndrome developed due to absolute or relative deficiency of insulin and it is mainly characterized by hyperglycemia [(1)]. In India currently 62 million diabetic individuals diagnosed with the disease and that's why it is noticed the fast-gaining status of potential epidemic. In 2000, India ranked first in the world by 31.1 million people with diabetes mellitus followed by China and United States with 20.8 million and 17.7 million people respectively.

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The Wild et al. predicted the prevalence of diabetes to double globally with a maximum increase in India from 171 million individual (in 2000) to 366 million individual in 2030[2].

Thus, so many synthetic drugs are available to treat the diabetes which is costly and giving serious side effects. To overcome this burden medical scientists are continuously in search of safe, cost-effective drugs to treat the diabetes.

The herbs have been used as food and medicine from ancient time; they are safe and cost effective [3].

Abutilon indicum (L.) Sweet belongs to the family Malvaceae. It is an important medicinal plant used in Ayurveda and is commonly called as Atibala. It is one of from the Balachatustayam. Acharya Bhavaprakash in Guduchyadivarga quoted Balachatustayam like 1) Bala 2) Mahabala 3) Atibala 4) Nagbala.

"बलामूलत्वचश्चूर्णंपीतं सक्षीरशर्करम्| मूत्रातिसारंहरति दृष्टमेतन्न संशयः||
हरेन्महाबला कृच्छ्रम् भवेद्वातानुलोमनी| हन्यादतिबला मेहं पयसा सितया समम्||"[4]

Botanically Sida have many species. But classically we are using these four in mutraatisar, mutrakruchh, amavat, anulomini, meha (diabetes mellitus) and as Rasayan respectively.

Atibala is well known to various tribal communities and forest dwellers. They use the various parts (leaves, root, seed and seed oil) for many diseases. In Melghat forest of Amravati district the tribal communities used the Atibala for diabetes mellitus [5].

The Atibala(Abutilon indicum Linn. Sweet) is easily available throughout India, having numerous properties by Ayurved and modern classics. The plant has rich source of major phytoconstituents' mucilage, tannins, β-sitosterol, asparagines, flavonoids, alkaloids, hexoses, nalkane mixtures, alkanol, gallic acid sesquiterpenes and has antioxidant, hepatoprotective, immunomodulatory, analgesic, anti-inflammatory, anti-arthritic, sedative, anticonvulsant, diuretic, wound healing, larvicidal, antimicrobial, anti-asthmatic, anti-diarrheal and anti-estrogenic activity[6].

According to Ayurved it is Rasayan, Balya, Bruhan, Vatahar and Kledashoshak property which are helpfulto treat the Vatajprameha(Madhumeha)[7]. Prameha, particularly Madhumeha and diabetes mellitus have similarity by etiological factors, clinical presentation and to some extent therapeutic aspects [8].

The root of Atibala (Abutilon indicum Linn Sw.) with Sita (sugar) and Godugddha (Cow milk) in equal quantity is taken for present PhD research work as the anti-diabetic study in STZ induced diabetic rats. Usually in diabetes mellitus the sugar and milk are not indicated. But Achyarya Bhavprakash mentioned in Guduchyadi varga with this anupan [4]. As the Diabetes mellitus is most considerable disease by ethical issues, this study is taken for animal experiment.

Present knowledge gap for stated problem:
In India anti-diabetic activity of Atibala, mostly leaves have been established in all study. Thestudy of Atibala root powder (churna) with equal quantity of sugar (sita) and Cow milk (Godugddha) is not carried out yetas per classic text, Bhavprakash nighantu in which it is mentioned to cure diabetes mellitus (Meha) completely [4].

The anti-diabetic activity of Atibala root with equal quantity sugar (Sita) and Cow milk (Godugddha) if proven in streptozotocin induced diabetic Albino Wister rats then it will be new effective remedy available for diabetes mellitus (Prameha particularly inMadhumeha) after clinical trial.

Most of the previous vivo studies have been carried out by using aqueous or alcoholic extract of Atibala leaves, while as according to Bhavaprakash Nighantu use of Atibala in the form of root powder with equal quantity of sugar (sita) and Cow milk (Godugddha) is effective for Diabetes mellitus. This knowledge gap will be fulfilled in my study.

Research question:
1. Whether the root of Atibala (Abutilon indicum Linn Sweet) with equal quantity of sugar (sita) and Cow milk (Godugddha) have the anti-diabetic activity?

2. Whether the root of Atibala (Abutilon indicum Linn Sweet) with equal quantity of sugar (sita) and Cow milk (Godugddha) is effective to control increased blood sugar level in Streptozotocin induced diabetic rats in comparison with Glimepiride in view of outcome as reducing blood sugar level?

Hypothesis:

Research Hypothesis:

The root of Atibala (Abutilon indicum Linn Sweet) with equal quantity of sugar (sita) and Cow milk (Godugddha) have exist the anti-diabetic activity in Streptozotocin (STZ) induced diabetic rats.

Null Hypothesis (H0):

The root of Atibala (Abutilon indicum Linn Sweet) with equal quantity of sugar (sita) and Cow milk (Godugddha) have not exist the anti-diabetic activity in Streptozotocin (STZ) induced diabetic rats.

Aims and Objectives:-

Primary objective:

1. To study anti-diabetic activity of Atibala root with equal quantity of sugar (sita) and cow milk (Godugddha) in Streptozotocin (STZ) induced diabetic rats.
2. To study the efficacy of Atibala root with equal quantity of sugar (sita) and cow milk (Godugddha) on increased blood sugar level in Streptozotocin (STZ) induced diabetic rats.

Secondary objectives:

1. To study the efficacy on the parameter like serum total cholesterol, serum triglycerides, serum high density lipoproteins when Streptozotocin (STZ) induced diabetic rats are treated with Atibala root with equal quantity of sugar (sita) and cow milk (Godugddha) at the end of study (21^{st} day)
2. To study the pharmacognostic, physico-chemical and phytochemical analysis of Atibala(Abutilon indicum Linn Sweet) root to assess the authenticity, purity and strength of the drug

Material and Methods:-

1. Study design: In vivo-Animal experimental study

2. Study setting:

Animal experimental work will be carried at approved Centre of animal studies.

Pharmacognostical & phytochemical analysis will be carried out at approved Centre.

Plan of work:

	Plan of work	
Pharmacognostic Study	Phytochemical study	Animal experimental Study
Collection of Root of Atibala	Collection of Root of Atibala	Study population
Sample authentication	Sample authentication	Sample size
Macroscopic evaluation	Sample drying	Sampling technique
Microscopic evaluation	sample powdering	Selection of study subjects
	physico-chemical evaluation	Operational definitions
	phyto-chemical evaluation	Test drug
		Dose selection
		Dose fixation
		Routes of administration
		Induction of diabetes
		Experimental study design
		Experimental procedure
		Anti-diabetic activity
		Method of measurement
		Method of data collection
		data analysis methods

Materials:-

The root of Atibala (Abutilon indicum Linn Sw) for the study will be collected in Shishir (late winter) as it is sheeta virya dravya [27]. It is collected from field.

Authentication

The authentication of Atibala (Abutilon indicum Linn Sweet) root sample will be done from approved Centre.

Pharmacognostic Study:

The study will be done according to API guidelines.

I. **Botanical evaluation**

1. Organoleptic characters
2. Macroscopic –
3. Microscopic
4. foreign matter

Phytochemical study:

I. Physico–chemical evaluation

1. Ash Value
2. Acid insoluble ash
3. Alcohol soluble extractive
4. Water soluble extractive

II.Phytochemical evaluation

1. TLC Qualitative
2. Determination of Phyto-chemical Constituents

III. Animal experimental study

The experimental study is designed to access the anti-diabetic activity of root of Atibala in STZ induced diabetic rats. With due permission of the Institutional Animal Ethics Committee (IAEC) the experimental work will be started.

The study will be conducted as per guidelines of CPCSEA (The Committee for the Purpose of Control and Supervision of Experiments on Animals)

A. Study population

Wistar strain albino rats of either sex having weight 150-200 gm. will be selected for the study.

B. Sample size determination

Sample size is determined with the help of 'Resource Equation method [28].This depends on law of diminishing returns. It needs an estimate of E

E= (total numbers of experimental units) – (numbers of treatment group)
And **E should be between10 to 20**
E is the number of degrees of freedom in an analysis of variance (ANNOVA).
It is based on the need to obtain an adequate estimate of the standard deviation.

In present study treatment group are 5. And if we consider sample size 4 then
E= 5*4 – 5 = 20-5= 15
E fall between10 – 20 therefore 4 animals per group are acceptable.
We will take 6 animals in each group by considering 20% dropout

C. Sampling technique:

The diabetic rats will be divided in to 5 groups and each group having 6 animals. The animal will be selected by simple random sampling.

D. Method of selection of study subjects:

Inclusion criteria:

Wistar strain albino rats of either sex weighing between 150-200g.

Healthy Albino Wistar rats having blood sugar more than normal will be selected for experiment [29].

Exclusion criteria:

1. Rats which are infected and pregnant will be excluded from the study.
2. Rats showing signs of infection during the course of study and those under other experiments will be excluded.

E. Operational definitions:

Anti-diabetic activity-Blood sugar lowering effect in STZ induced diabetes in Albino Wister rats.

Atibala root powder with vehicle- Sugar [Rock sugar (KhadiSakhar)] and Cow milk (Godugddha) is used as vehicle with Atibala (Abutilon indicum Linn Sw.)

F. Dose selection:

According to database on medicinal plant used in Ayurveda human Atibala root dose is 5 to 10 gm [7]. In one research paper the effective dose of Atibala 10gm is mentioned for the decoction, so the dose of Atibala powder for present study is converted from human dose of Atibala powder 10 gm [30].

G. Dose fixation:

The dose is calculated by extrapolating the human dose to animal based on the body surface area ratio by referring to the table of Paget and Barnes [31]. Conversion formula: = Human Dose × 0.018 (conversion factor for Rats) Test drug

Atibala powder = Human Dose × 0.018 (conversion factor for Rats)

=10 × 0.018

= 0.18/200g body weight of Rat

=0.9gm/kg.

Reference standard drug – Glimepiride [29]

= Human Dose × 0.018 (conversion factor for Rats)

= 1 × 0.018 = 0.018 mg/200g body weight of Rat

= 0.09 mg/kg

H. Route of administration:

First mix Atibala fine powder into equal quantity sita(sugar) and Godugddha (cow milk) to make suspension and administered orally in animals with the help syringe. Dose will be calculated according to body weight of animals. The reference standard drug Glimepiride will be given orally by dissolving it in distilled water [32].

I. Induction of Diabetes:

Diabetes will be induced in albino rats by single intraperitoneal injection of streptozotocin as per dose 60mg/kg. Streptozotocin will be weighed for individually for each animal. According to its weight it will be solubilized with 0.2 ml saline (154 mMNaCL) just prior to injection. After 72h of streptozotocin injection, rats with diabetic hyperglycemia (blood sugar level more or equal to 250 mg/dL) will be selected for experiment [31].

J. Animal experimental study design:

S. No.	Group Name	Group Code	Sample size	Intervention/Drug	Route of Drug Administration
1.	Normal Control	Group A	6	Distilled Water	Oral
2.	Standardcontrol	Group B	6	Glimepiride	Oral
3.	Atibala root powder	Group C	6	Atibalaroot powder	Oral
4.	Atibala root powder + Vehicle	Group D	6	Atibala root powderwith sugar(sita) & Cowmilk (Godugddha)	Oral
5.	Vehicle control group	Group E	6	Sugar(sita) & Cow milk (Godugddha)	Oral

K. Experiential Procedure :[23]

Test drugs will be administered for 21 consecutive days. The random blood glucose level will be measured at 0, 7th, 14th and 21th days of drug administration by using one touch glucometer. Tail vein puncture method will be applied for collection of blood sample in the rats. On 22th day animals will be sacrificed by administering overdose of ether anesthesia. The blood will be collected by dissection of jugular vein and forwarded to biochemistry laboratory for assessment of biochemical parameters. The animals will be autopsied and the pancreas will be sent for histopathological study regarding Anti-diabetic activity[31].

Anti-diabetic activity will be evaluated by the effort of test drugs on biochemical parameters. i) Biochemical variables: blood sugar level (0-day, 7th day, 14th and 21th day), serum total cholesterol (0 day and 21th day), serum triglycerides (0 day and 21th day), serum high density lipoproteins (0 day and 21th day), blood urea (0 day and 21th day), serum creatinine (0 day and 21th day), Serum glutamic oxaloacetic transaminase (SGPT) (0 day and 21th day). ii) Histopathological study: pancreas (22th day)

	Group A				Group B				Group C				Group D				Group E			
DAY	0	7	14	21	0	7	14	21	0	7	14	21	0	7	14	21	0	7	14	21
Body Wight																				
Blood sugar																				

	Group A		Group B		Group C		Group D		Group E	
DAY	Before	After	Before	After	Before	After	Before	After	Before	After
serum total cholesterol										
serum triglycerides										
serum high density lipoproteins										

L. Methods of measurement:

Weighing scale will be used for measurement of weight of rats. Glucometer with available strips will be used for the measurement of random blood sugar level. The auto analyzer will be used for estimating different biochemical parameters. (References given in the kit literature mentioning the basis of the methods on which test procedures will be evolved) and histopathological changes in pancreas will be measured by microscope.

M. Methods of data collection:

Data will be collected from the analysis reports of random blood sugar, biochemical parameters and histopathology.

N. Data analysis methods:

Statistical significance will be analyzed using student't' test, repeated measure ANNOVA test. The results will be considered on 95% confidence limit. All the statistical analysis will be performed using statistical software Epi Info 7.2.2.1 (year2017).

Observations:-

Observations will be recorded and results will be obtained on the basis of appropriate statistical tests at the end of the experimental study.

Discussion & Conclusion:-

The results obtained from study will be discussed. The work will be summarized and the final conclusion will be drawn.

Ethical considerations:

With the clearance of the Institutional Ethical Committee (IEC) of concerned institute &Institutional Animal Ethics Committee (IAEC) of approved Centre, the experimental work will be commenced.

The study will be conducted as per guidelines of CPCSEA (The Committee for the Purpose of Control and Supervision of Experiments on Animals)

Generation of new knowledge:

In asthenic type of diabetic patient, the treatment should be mainly based on the line of increasing stamina and vitality by way of tonic (Bruhan) drug [33]. In diabetes medicines have to use long term with no complication. Atibala root is the Rasayan drug. It can use in long term. It is useful in diabetic neuropathy also [30]. So, it is decided to study the anti-diabetic activity of Atibala root with anupan sugar and cow milk as per classic reference.

Cow milk (Godugddha) and sugar(sita) are supposed to be contraindicated in established treatment available for treating diabetic mellitus due to the most considerable disease by ethical issues, so this animal experiment study will be newly established concept in treating diabetes mellitus by Atibala root with anupan sugar(sita) and Cow milk(Godugddha). This is the generation of new knowledge from my study to treat the asthenic type of diabetic patients.

Abbreviations:

1. API- Ayurvedic Pharmacopeia of India
2. AR-Atibala root
3. Arv-Atibala root +vehicle
4. BP- Bhavaprakash
5. CD- Chakradatta
6. Ci- Chikitsa
7. CS- Chararksamhita
8. DC (STZ) – Diabetic Control (Streptozotocin)
9. NC - Normal Control
10. SC- Standard control
11. STZ - Streptozotocin
12. VC- Vehicle control

References:-

1. Davidson S. Principles and practice of medicine. 17th ed. London, England: Churchill Livingstone; 1995,724.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004 May;27(5):1047-53.
3. George P. Concerns regarding the safety and toxicity of medicinal plants-An overview. Journal of applied pharmaceutical science. 2011 Jan 1;1(6):40-4.
4. Sri BramhasanshakarMisra and ShriRupalaljiVaishya. Guduchyadivarga. Bhavaprakshnighantu.Chaukhambha Sanskrit sansthana, Varanasi,1999:9 Edition:367
5. Sarkardm, sarkar um, mahajan nm. Anti-diabetic and analgesic activity of leaves of abutilon indicum. Asian journal of microbiology biotechnology and environmental sciences. 2006;8(3):605.
6. Raut S, Bandawane d. Pharmacognistic and pharmacological aspect of abutilon indicumlinn: a review.
7. Sharma P.C.,Yelne M.B., Dennis T.J., Database on Medicinal plants, CCRAS New Delhi, Vol.2:2005; P. No51.
8. Murthy AR, Singh RH. Concept of prameha/madhumeha (contradictions and compromises). AncSci Life. 1989 Oct;9(2):71-9.
9. Ayurved Pharmacopeia of India, Government of India,Ministry of health and family welfare Dept of Ayush New Delhi, Part1;Vol.1, First edition,P.N.25-26.
10. DrIndradevTripathi, Chakradatta Of shriChakrapanidatta, Chaukhamba Sanskrit SansthanVaranasireprint edi.2005, P. No.207.

11. Shri Mishra BramhashankarShastri, Bhavaprakasha, Vidyotini Hindi commentary; Part II,Chaukhambha Sanskrit sansthana, Varanasi,19 edition 2005,P.N.358.
12. Shri Mishra BramhashankarShastri, Bhavaprakasha, Vidyotini Hindi commentary; Part II ,Chaukhambha Sanskrit sansthana, Varanasi,19 edition 2005,P.N.762
13. Pt.KashinathaShatri and DrGorakhanathaChaturvedi,CarakaSamhita of Agnivesha with introduced by ShriSatyanarayanShastri, ChaukhambaBharati Academy, Chikitsasthan: Reprint 2019;P.No27.
14. Pt.KashinathaShatri and DrGorakhanathaChaturvedi,CarakaSamhita of Agnivesha with introduced by ShriSatyanarayanShastri, ChaukhambaBharati Academy, Chikitsasthan: Reprint 2019;P.No27.
15. Vd. YadavajiTrikamajiAcharya and Narayan Ram Acharya, 2002 7th publication, sushrutasamhita of sushruta with the Nibandhasangraha commentary of ShriDalhanacharya and Nyayachandrikapanjika of shriGayadasacharya on Nidanasthana; Chaukhmbhaorientalia, Varanasi. Reprint 2012, P.N.499
16. DrMadhaw Singh Baghel and Dr Girish KJ,researches in Ayurveda-Directory of Titles of All India Ayurvedic PG And Ph D Theses (Third / Web Edition)
17. Krisanapun C, Lee SH, Peungvicha P, Temsiririrkkul R, Baek SJ. Antidiabetic activities of Abutilon indicum (L.) Sweet are mediated by enhancement of adipocyte differentiation and activation of the GLUT1 promoter. Evidence-Based Complementary and Alternative Medicine. 2011 Jan 1;2011.
18. Krisanapun C, Peungvicha P, Temsiririrkkul R, Wongkrajang Y. Aqueous extract of Abutilon indicum Sweet inhibits glucose absorption and stimulates insulin secretion in rodents. Nutrition research. 2009 Aug 1;29(8):579-87.
19. Sarkardm, sarkar um, mahajan nm. Anti-diabetic and analgesic activity of leaves of abutilon indicum. Asian journal of microbiology biotechnology and environmental sciences. 2006;8(3):605.
20. Seetharam YN, Chalageri G, Setty SR. Hypoglycemic activity of Abutilon indicum leaf extracts in rats. Fitoterapia. 2002 Apr 1;73(2):156-9.
21. Adisakwattana S, Pudhom K, Yibchok-Anun S. Influence of the methanolic extract from Abutilon indicum leaves in normal and streptozotocin-induced diabetic rats. African Journal of Biotechnology. 2009;8(10).
22. Bolleddu R, Venkatesh S, Rao MM, Shyamsunder R. Anatomical and antihyperglycemic activity of Abutilon indicum L. Sweet (Atibala) fruits.
23. Kaushik P, Kaushik D, Khokra SL, Sharma A. Antidiabetic activity of the plant Abutilon indicum in streptozotocin-induced experimental diabetes in rats. Int. J. Pharmacog. Phytochem. Res. 2010 Aug 30;2:45-9.
24. Krisanapun C. Hypoglycemic Effect and Mechanisms of Action of Extract from Abutilon Indicum Sweet.
25. Kumar ph, kondamounika p, kavya e, girisha s. Anti-hyperglycemic activity of abutilon indicum using alloxan induced hyperglycaemia in mice.
26. Adisakwattana S, Pudhom K, Yibchok-Anun S. Influence of the methanolic extract from Abutilon indicum leaves in normal and streptozotocin-induced diabetic rats. African Journal of Biotechnology. 2009;8(10).
27. Pt.KashinathaShatri and DrGorakhanathaChaturvedi, Caraka Samhita of Agnivesha with introduced by ShriSatyanarayanShastri, ChaukhambaBharati Academy, Kalpasthan: Reprint 2007;1/10,P.No27.
28. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR journal. 2002 Jan 1;43(4):244-58.
29. Khedekar S, Rukkudin G, Ravishankar B, Prajapati P. Anti-diabetic activity of traditional Indian gold containing preparation: ShadgunaBalijaritaMakaradhwaja on streptozotocin induced diabetic rats. J InterculEthnopharmacol. 2016 Feb 21;5(2):162-7.
30. Patel K, Patel M, Gupta SN. Effect of Atibalamula and Bhumyamalaki on thirty-three patients of diabetic neuropathy. Ayu. 2011 Jul;32(3):353-6.
31. Paget GE. Evaluation of Drug Activities. Pharmacometrics. 1964.
32. Bramhanandtripathi, Sharangdharsamhita, Madhyamkhand, shashtoadhhya. 1998(3):6:173)
33. Srinivas P, Devi KP, Shailaja B. Diabetes mellitus (madhumeha)-an Ayurvedic review. Int J Pharm Pharm Sci. 2014;6(Suppl 1):107-10.