VOLUME - 10, ISSUE - 03, MARCH - 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Diabetology

AN ANIMAL STUDY FOR ANTI DIABETIC ACTION OF POLY- HERBAL DRUG

Dr. Canchi Bhoopal Dhanraj	MD (AY)(OSM) MPH (ENGLAND) (Ph.D)(UHS), Professor, Bharat Ay, Medical College, Muzaffar Nagar –UP.
Dr. Gajji. Babu*	MD, (AY), Ph.D (RAU) Jodhpur, Director, C.Ay.R.I, Ministry of Health, AYUSH, Govt. of India, Jhansi –UP. *Corresponding Author
Dr. Ratnakaram Vidyanath	MD (AY), Ph.D (UHS), Professor-Samhitha (Rtd.). Dr.BRKR Govt. Ay. Medical College, Hyderabad.
Dr. Gottumukkala Krishna Mohan	M.Pharma, Ph.D, Professor & Director I.S.T. J.N.T. University, Hyderabad.
Dr. S. Renuka Rani	MBBS. MD., DCP.UHS), Dy,civil Surgeon (Rtd), S.R.R.I.T.C.D, Hyderabad.
Dr. Mondi Sandhya Rani	M.Pharma Ph.D, Assistant Professor(c), Centre for Pharmaceutical Sciences, Institute of Science and Technology J.N.T.U., Hyderabad.
Samanthula Vijay Kumar	M.Pharma. Asst. Professor VIPER Narsapur.

ABSTRACT

Aim: To compare the efficacy of the selected poly herbal medicine for anti-diabetic activity. ***Methods and Materials:*** Healthy thirty six Albino wistar rats are selected and divided into six groups of six each. named as ABCDEF. A group animals as control group without administering the research drug. B group as Standard group animals and were administrated Glibenclamide 10 mg/kg BW..Screening methods for anti diabetic activity are:a)Acute toxicity studies :. The animals of group C,D,E,F are given research drug in different doses and are observed for 48 hours for toxicity effects. b) Normoglycemia—Blood samples were collected through retro-orbital puncture at 0h, 2h, 4h, and 6h after dosing, for determination of blood glucose levels and the blood insulin levels.c) Euglycemic studies: The animals were induced diabetes with Intra peritoneal injection of Streptozotocin 60 mg/per kg body weight and after 48 hours the animals are given the research drug in different dose to Diabetic induced rats. the blood sugar and the blood insulin levels are measured. the study is divided into two phases. A) Short term study , for this group of overnight fasting animals the research drug was given on day one only and samples of blood collected from retro-orbital veins on zero hour at frequent intervals up to 24 hours. B)Long term study.The drug is administered for 14 days, samples are drawn on daily basis and compared with the standard drug group blood samples. After the study one animal from control group, standard group, and F group was selected and sacrificed and pancreas and liver have been taken for histopathological examination. **Results:** There was no mortality of animals in toxicity studies. Statistical analysis was done with one way ANNOVA and found the drug was effective to reduce the blood sugar level with ($P<0.001$) and increase in Blood insulin level with ($P<0.001$) compared to the standard drug.

KEYWORDS : Glibenclamide, Intra peritoneal, Streptozotocin, retro-orbital.

INTRODUCTION

The first physicians to identify the disease was Charka (1000BC) and Sushrutha 6th Century BC as Madhumeha. The Diabetes word was coined by Apollonius, medical text written around 1425, in 1675 Thomas Willis added Mellitus to Diabetes[1].

The metabolic disease or Diabetes Mellitus or Madhumeha is a condition caused due to body"s infective use of or lack of insulin. 422 million people world wide have Diabetes[2]. The Global prevalence of Diabetes Mellitus for global population over age of 18yrs was 4.7% in 1980 and increased to 8.5% in 2014[3]. India has nearly 33 million diabetic subjects today, which is briefly contributed by the urban population. Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population worldwide[4] and a key issue of concern.

The disease in its severe state affects major systems of the body, leading to multi-organ complications. The other predisposing causes could be due to excessive body weight or lack of physical activity. The types of diabetes are :1.Type I Diabetes, 2.Type II Diabetes Mellitus, 3.Gestational Diabetes.

Oxidative stress in Diabetes mellitus plays a major role in pathogenesis of the diseases, the same established in Ayurveda as vitiation of Dathus by vata is the cause for conversion of Pramehas to Madhumeha /Diabetes Mellitus[5]. Diabetes increases oxidative stress in many organs, especially in the liver[6,7].Liver is one of the most important organs that maintains blood glucose levels within normal limits thus enhancement of blood sugar yield to imbalance of oxidation-reduction reactions in hepatocytes, so that, hyperglycemia through increasing in AGEs (advanced glycation end products) facilitates free radicals production via disturbance in ROS (reactive oxygen species) production . Early stages of diabetes, tissues injuries are induced via hyperglycemia but its progress in latter stages is not related to hyperglycemia. Therefore, monitoring of blood glucose levels solely is not sufficient in retarding diabetes complications. Thus, a suitable drug must have both antioxidant and blood glucose decreasing properties, with the same principle this drug combination has been chosen[8 to 13]. An ancient physician Vagbhatta of 3 rd century says that all conditions where urine resembles honey in all aspects and even the body becomes sweetish, should be regarded as Madhumeha[14,15,]. Madhava kara in his treatise Madhvanidhana describes that Prakarasena Prabhutam Prachuram varam varam karothi Ethi Prameha[16,17]. It means a disease in which large amount, turbid , urine is frequently excreted such an disease is called as Prameha/ Diabetes mellitus. Same was told by

Vagbhatta[18]. Charka an ancient physician describes that all diets and behaviours which attribute to increase Kapha will indirectly cause Madhumeha Diabetes mellitus[19]. He also expressed that this disease could also be because of genetic cause , and expressed as Beeja doshaja and Kula Doshaja[20].

The most important complication of Diabetes Mellitus are cardiovascular, kidneys, nerves, foot ulcers, infections and eyes .2.6% Global blindness is due to diabetes[20]. The common symptoms of diabetes are polydipsia, Polyuria, constant hunger, weight loss, fatigue and vision changes. Oral hypoglycemic agents like sulphonyl-ureas and bi-guanides are the conventional drugs used for the treatment, but the adverse side effect associated with these drugs is a major limitation[21,22].

The herbal medicines are becoming popular due to better results and safe use as compared to marketed drugs and more effective treatment of health problems[23]. Plants possessing anti-diabetic activities are of significant interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts and a number of them have shown varying degree of hypoglycemic and anti-hyperglycemic activity[24]. The bioactive constituents found in many plant species are isolated for direct use as drugs, lead compounds, or pharmacological agents. These traditional approaches might offer a natural key to unlock diabetic complications[25] . The chemical structures of a phytomolecule play a critical role in its antidiabetic activity. The efficacy of hypoglycemic herbs is achieved by increasing insulin secretion, enhancing glucose uptake by adipose and muscle tissues, inhibiting glucose absorption from intestine and inhibiting glucose production from hepatocytes[26] .

The conversion to diabetes is enhanced by the low thresholds for the risk factors, such as age, body mass index and upper body adiposity. Indians have a genetic phenotype characterized by low body mass index, but with high upper body adiposity, high body fat percentage and high level of insulin resistance[27].

Plants have been used to treat diseases such as diabetes, jaundice, cardiovascular diseases, heavy metal poisoning, congestion of abdominal and pelvic cavities and scarlet fever[28]. It is estimated that out of 250,000 to 500, 000 species of plants only 1 to 2% of the terrestrial plants have been reasonably well investigated. Although today the synthetic drugs are larger in their number than the natural ones but still many synthetic drugs have their origin in the natural source and have been derived from plants and animal[29].

Structurally different compounds may be present in the plant which may have synergic effect. Complexity of these drugs and their biological variations make it necessary to evaluate their safety. Therefore, this study has been designed to understand the synergetic action of this herbal compound drug which is easily available through out India at low prices and can be prepared at home with out any big equipment by a common man. Added to it, this medicine comprises of simple herbs and fruits which could be procured without cutting the big trees which helps for environmental safety compared to the other products of the market. The above facts of Diabetes Epidemiology in India and globally, and non-availability of proper drugs which are natural, freely available economic herbs should have a proper drug combination without side effects has pushed to explore the synergetic action of this compound herbal drug (Guduchyadhi Choornam) which is hypoglycaemic and anti-Oxidant for treatment of type II Diabetes Mellitus.

AIM OF STUDY:

To **compare the efficacy** of the selected poly herbal medicine for anti-diabetic activity with a control and standard anti diabetic drug in animals.

METHODS AND MATERIALS:

Rationale for animal usage: Pre-clinical studies Provide general profile of pharmacological action pharmacokinetic and toxicity of new drug. So, before the administration of new drug to human being it is necessary that pre-clinical studies are carried out in smaller animals like rats to understand their disposition. Both the International Drug Regulatory Authorities and the Drug Control Authority of India requires the generation of toxicity data for drugs in 2 species of rodents. Therefore, all R & D units of Pharmaceutical companies and also Contract Research Organizations in the country use rodents (mice and rats) for generating data. The most commonly used species are the rats and currently Albino Wistar rats are being used for pharmacological and toxicity data generation. Studies reported in the literature on anti-diabetic activity have also been conducted on Albino Wistar rats. It is therefore proposed to use Albino wistar rats for these studies as the data generated would be directly useful as reference data for all future studies. Regulatory requirements necessitate conduct of anti-diabetic studies. Though the herbal drugs are abundantly in use, but to be more on safer side for the synergetic action of the compound drug the toxic studies and hypoglycemic studies will be done on the Albino wistar rats.

ANIMALS: The animal studies have six groups. One is control group (In this group no test drug is given) and the other is Standard group that is the animals are given Standard drug that is Glibenclamide. The test goup have four groups. C,D, E ,F. In CDEF groups the animals are given test drug in different doses 500mg,1000mg,1500,2000mg/kg body weight respectively.

The present study was done on healthy adult albino rats in the weight range of (150-240 gm), selected from an inbred group housed in specially designed cages and maintained under standard conditions of temperature (23 ± 1°C) and humidity of (55-60%) with a 12-hour light and 12-hour dark cycle for at least one week before use. To analyze statistically each group should contain minimum six animals each.. Total of six groups thirty six animals were used.

All animals consumed standard rodent diet and tap water ad libitum. Clinical monitoring of the animals was also performed to evaluate body weight and blood glucose and insulin levels weekly. All animals were cared for according to the guiding principle in the care and use of animals[30].

SCREENING METHODS FOR ANTI-DIABETIC ACTIVITY A) Acute toxicity studies B).Normoglycemia:Assessment of hypoglycemic activity in normal healthy rats. C) Euglycemic studies: Diabetic induced rats.

Aims & Objectives of Toxic studies: 1.To determine if there is any acute toxic effect of research drug on the Albino rats by oral administration. 2. To study the effect compound herbal drug (Combination of 10 herbal drugs) for anti-diabetic activity using Steptozotocin induced diabetic model,. compared with a standard drug (Glibenclimide).

I) ACUTE TOXICITY STUDIES IN ANIMALS Acute toxicity study was carried out according to method described in OECD guidelines-425. Guduchyadhi choornam (composition of 10 herbal drugs) was suspended in 1% carboxylmethyl cellulose in doses of 200mg, 500mg, 1000mg, 1500mg and 2 g/kg body. wt were each group administered orally to albino rats of either sex 150-240g The animals were observed continuously for any change in behavioral responses for first few hours and later 24 hours intervals for a period of 48hours.at the end of this period, the mortality if any was noted[31,32].

II) NORMOGLYCEMIC STUDIES Assessment of hypoglycemic

activity in normal healthy rats.Carry out the study with different doses of the Guduchyadhi choornam (GC) in normal healthy rats fasted overnight. Divide the animals into groups of six animals each and administer the test drugs per orally with the vehicle in different doses to different groups of animals . Group I - Control, 1% Carboxy Methyl Cellulose suspension in water.Group II - Standard, Glibenclamide, 10 mg/kg b.w, p.o[33].

To see Hypoglycemic activity: Group III & IV and V & VI- The powder of the poly herbal drug with the vehicle in different doses Group III to group VI) as fixed after the toxicological studies respectively. Blood samples to be collected through retro-orbital puncture at 0h, 2h, 4h, and 6h after dosing, for determination of blood glucose levels from the rats and the blood insulin levels. The glucose levels and blood insulin levels are compared with the drug dose levels and the inference is made regarding the hypoglycemic activity.

III. EUGLYCEAMIC STUDIES Induction of diabetes: Induction of diabetes was done as described by Trivedi et al., (2004). Animals were allowed to fast overnight prior to injection. Hyper-glycemia was induced in overnight fasted albino rats of albino wistar strain (150-240gm)[34]. Thirty six normal healthy rats are selected and put into six groups comprising of six each. All animals are weighed and list is prepared (enclosed). Animals are fated for 8 to 12 hours before administration of STZ injection Intra-peritoneal.Five percent dextrose solution is prepared to be given to the animals soon after the administration of STZ Streptozotocin injection given. Buffer solution for dissolving the STZ is prepared.STZ is added to the buffer solution. Animal weight chart with STZ dose chart was prepared and Administered drug accordingly.

Induction of diabetes was done as described by Trivedi et al., (2004)[35]. Animals were allowed to fast overnight prior to injection. Hyperglycemia was induced in overnight fasted albino rats of Albino wistar strain (150-240 gm). A single intraperitoneal injection of freshly prepared Streptozotocin (STZ) stored at 4°C temperature and protected from environmental extremes (STZ; 60 mg/kg B.wt.) dissolved in freshly prepared 0.05 M of sodium citrate buffer, pH=4.6 [18]. Fasting rats must measure their normal blood glucose level before STZ injection (zero-time), then measured again after 48 hr of STZ injection to be sure that the rats became diabetic, their weight also must be determined before and after injection[36].

Steptozotocin (60 mg/Kg body weight) was given within 50-75 seconds. The rats were kept on 5% glucose solution in the cages to prevent hypoglycemia were selected for the anti-hyperglycaemic study after 2 days /48 hours. The blood glucose level was determined by glucose oxidase method using a one touch basic plus glucometer.

After induction of Diabetes in overnight fasted rats, Fasting blood glucose (FBG) levels of the rats were observed to select the diabetic rats for the experiment. FBG levels of rats ranging from >250mg /dl was selected and divided arbitrarily in to groups of six rats in each group and drugs administered to respective groups. Group I (Control) vehicle(1% Carboxy Methyl Cellulose suspension in water).Group II (Standard) (Glibenclamide 10 mg/kg b.w, p.o).Group III to VI test drug Guduchyadhi choornam in different doses is administered with vehicle.

Short term study: Blood samples were collected at 0h, 2h, 4h, and 6h, 8h,12h,24h. after dosing for determination of blood glucose levels(BGL) and blood insulin levels(BIL) from the rats on the 0 day only.

Long term study: All the animals of all groups are given the respective control standard and testdrugs from '0'day to 14 th day. On ZERO day before giving the respective substances, the blood samples are collected, then blood samples are collected on 1 st day, 2 nd day, 4 th day, 7 thday and 14 th day, from retro-orbital plexus of animals of each group for BGL and BIL. On the above days the rats are put for over night fasting for 16 hours before drawing the blood and also post prandial blood samples are collected[37].

In all these experiments, approximately 0.5-1 ml blood was withdrawn each time from theretro-orbital plexus and separate serum immediately by centrifuging at 3000 rpm for 30 min for laboratory insulin estimation samples. Analyzed the glucose concentration in the serum samples immediately by the glucose oxidase(GOD-POD) method using Glucose Kit.The blood glucose levels and blood insulin are compared with statistical tools and the outcome would be determined, by using SPSS statistical soft ware.

HISTOPATHOLOGICAL EXAMINATION: After completion of biological fluid examination of the animals, one animal from each group is selected, i.e., control group, standard group, and from the last group of test drug i.e., group F , by following the standard proceedures the animals are sacrificed . The sacrificed animals were quickly dissected. Sample of the liver and pancreas were removed and and were preserved in formalin for Histopathological examination[38,39].

STATISTICAL ANALYSIS OF RESULTS

TOXICITY STUDIES RESULTS: It is found that all the animals were active and no mortality was found. So the experimental drug is biologically safe for human use.

The results were given in terms of Mean ± SEM. The obtained results were statistically analyzed utilizing one way analysis ANOVA using computerized Graph pad Instat version 3.05 and were considered statistically significant when P<0.05.

Table 1.LONG TERM STUDIES (BGL)

Gp	0DAY	1st DAY	2nd DAY	4th DAY	7th DAY	14th DAY
A	418.3±5 1.71	401.7±5 9.82	362.3±5 2.18	246.3±9 6.62	381.8±5 8.52	407.8±4 4.94
B	269.0±5 5.81	241.2±6 1.74	236.2±5 9.42	281.0±6 5.10	193.8±5 5.61*	175.3±3 8.97*
C	412.5±3 2.50	413.7±2 9.01	386.0±2 6.16	345.5±2 1.57	289.5±1 6.91**	196.2±1 3.82***
D	521.7±2 7.86	504.7±2 9.01	480.5±2 4.18	375.2±4 1.23**	325.0±3 6.40***	203.7±7 .149***
E	492.0±5 1.23	456.2±4 9.78	426.5±4 3.76	348.7±3 1.77*	283.8±2 4.78***	184.5±6 .989***
F	595.0±3 .416	515.0±2 9.15*	486.8±2 3.98**	415.2±1 6.50***	328.2±1 7.21***	177.0±7 .330***

Values are given as Mean± S.E.M (n=6);* p<0.001;** p<0.01;*p<0.05 compared with control.** In long term studies performed for 14 days .The results showed significant(p<0.0001) reduction of blood glucose levels at all doses of churna C,D,E,F as 29.81% , 37.70% ,42.31% , 44.84% respectively on 7th . Among all better reduction was found at a dose of F (70.25%) which was found to be greater to the reduction found with standard at a dose of 10 mg/kg b.wt on same 14thday (34.8%).

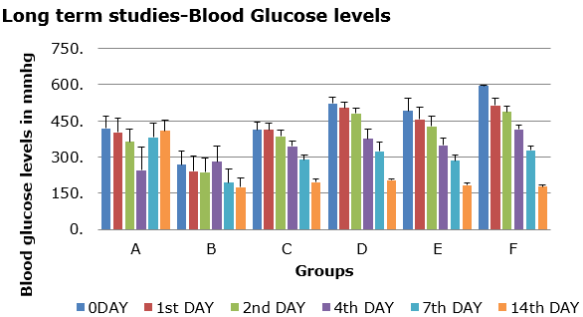


FIGURE:LONG TERM STUDIES (BGL)

Table 2SHORT TERM STUDIES (BGL)

	0HR	2HR	4HR	8HR	12HR	24HR
A	386.3±37.37	373.3±35.32	358.7±34.41	352.7±33.02	340.5±32.10	329.0±32.32
B	135.7±7.779	108.0±3.130**	91.17±6.570***	87.00±5.865***	87.17±2.315***	79.17±3.291***
C	420.5±11.27	390.8±15.08	371.7±16.26	311.3±34.88	289.3±44.90*	270.5±42.63**
D	343.3±45.71	289.7±37.30	257.2±39.88	255.7±43.05	260.8±45.94	261.3±49.63
E	501.8±52.10	490.8±54.50	407.8±27.43	383.8±24.56	369.2±25.52	350.3±23.18*
F	585.2±14.83	583.3±10.54	553.5±36.71	518.5±29.72	490.8±39.53	423.5±33.78**

Values are given as Mean± S.E.M (n=6); ***p<0.001; **p<0.01; *p<0.05 compared with control

The results showed significant (p<0.01 and p<0.001) reduction of blood glucose levels at 24hrs period with percentage reduction of 35.67%,23.8%,30.19% and 27.63% for C,D,E and F respectively, which was comparable to the reduction in glucose level 33% at 4th hr and reached 35.55% at 8th hr itself when standard glibenclamide was used with a significance of (p<0.0001)

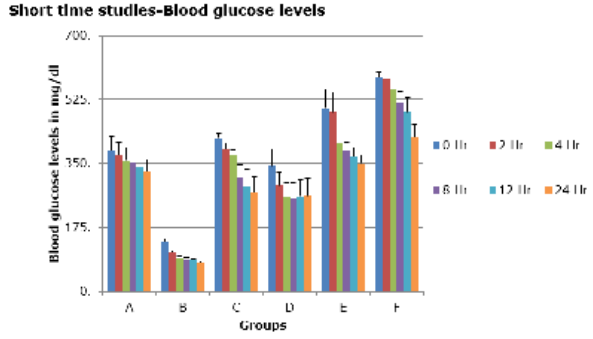


FIGURE 2HYPOGLYCEMIC STUDIES (BGL))

Table 3 NORMOGLYCEAMIC STUDIES(BGL)

	0HR	1HR	2HR	4HR	6HR
A	117.0±6.132	113.7±5.610	109.5±4.593	107.0±4.858**	103.8±4.491***
B	132.3±2.342	114.8±3.764*	102.2±2.041***	94.17±2.639***	85.50±1.871***
C	141.5±15.28	132.5±12.01	125.5±10.23	115.8±6.824**	109.7±13.72***
D	128.0±10.53	119.7±11.64	107.0±17.78	100.3±16.01**	95.00±12.70**
E	130.2±7.885	118.5±5.206	106.3±9.953***	96.83±10.30***	90.17±11.79***
F	121.5±5.541	113.0±5.933*	92.83±3.710***	80.83±5.076***	71.17±5.845***

Values are given as Mean± S.E.M (n=6); ***p<0.001; **p<0.01;*p<0.05 compared with control

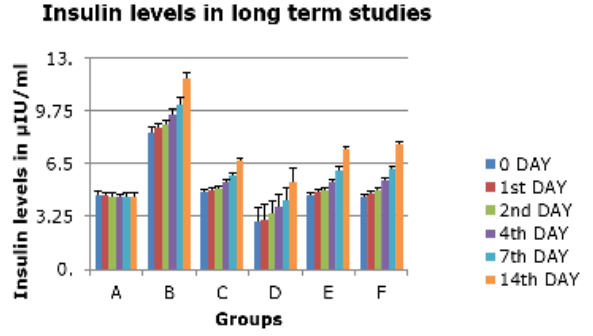


Figure3.long term studies (BIL)

Table 4 LONG TERM STUDIES (BIL)

Groups	0DAY	1st DAY	2nd DAY	4th DAY	7th DAY	14th DAY
A	4.543±0.277	4.533±0.1922	4.495±0.1861	4.475±0.1775	4.505±0.1764	4.487±0.2037
B	8.447±0.3146	8.665±0.3058	8.882±0.3113	9.493±0.4030***	10.17±0.4325***	11.71±0.4423***
C	4.723±0.1538	4.860±0.1436	4.998±0.1329	5.395±0.1297*	5.788±0.1312***	6.662±0.1903***
D	2.892±0.9172	3.077±0.9237	3.417±0.7984	3.800±0.8107	4.242±0.8207	5.405±0.7855
E	4.582±0.1331	4.792±0.1335	4.842±0.1706	5.382±0.1630*	6.110±0.2254***	7.368±0.2050***
F	4.447±0.1284	4.665±0.1248	4.882±0.1271	5.493±0.1645***	6.167±0.2254***	7.705±0.1806***

Values are given as Mean± S.E.M (n=6); ***p<0.001; **p<0.01;*p<0.05 compared with control

Long term insulin levels: In diabetic rats among all doses of churnam.

Among insulin levels found to show significant increase on 14th day(p<0.01).Greater improvement in insulin secretion levels on 14th day (p<0.001) which was comparable to that of standard drug glibenclamide at a dose of 10 mg/kg b.wt on 14th day.

TABLE5 SHORT TERM STUDIES (BIL)

	0HR	2HR	4HR	8HR	12HR	24HR
A	4.518±0.1970	4.563±0.1994	4.672±0.1946	4.758±0.2035	4.945±0.2556	4.928±0.3052
B	6.780±0.1396	7.063±0.7859	7.795±0.9712	8.122±1.139	8.483±1.034*	9.138±1.220***
C	4.835±0.06004	4.903±0.05011	4.940±0.04626	4.998±0.03781	5.057±0.03412**	5.130±0.02944***
D	2.892±0.9172	2.935±0.9302	2.960±0.9383	3.023±0.9562	3.003±0.9551	3.135±0.9904
E	4.582±0.1331	4.632±0.1295	4.887±0.2427	4.932±0.1007	5.083±0.1138	5.483±0.1619**
F	4.537±0.1210	4.897±0.1682	5.128±0.2183	5.455±0.2894	5.650±0.3105*	6.022±0.3717**

Values are given as Mean± S.E.M (n=6); ***p<0.001; **p<0.01;*p<0.05 compared with control

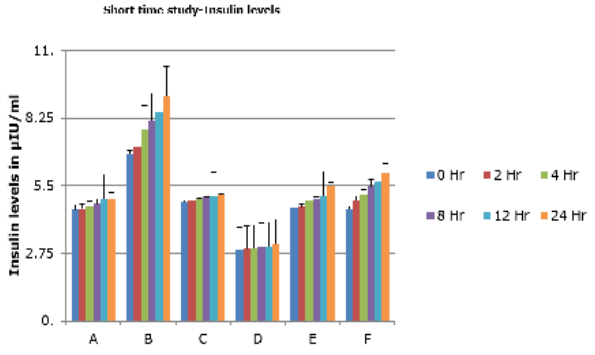


FIGURE 4.SHORT TERM STUDY (BIL)

In diabetic rats serum insulin levels found to increase significantly after 12 hrs,but drug c and F found to show response with significant (p<0.001)improvement in insulin levels which was comparable to standard glibenclamide in 24 hrs

Table6 NORMOGYCEMIC STUDIES (BIL)

	0HR	1HR	2HR	4HR	6HR
A	10.97±0.9861	11.05±0.9920	11.11±0.9937	11.23±0.9886	11.40±0.9114
B	12.60±0.7038	13.23±0.8042	14.17±0.6280**	15.06±0.8666***	16.24±0.9057***

C	12.57±0.7005	12.89±0.7908	13.65±0.3762*	14.01±0.6727**	14.69±0.3672**
D	12.45±0.5080	12.92±0.4624	13.24±0.4056*	14.03±0.5514***	14.77±0.3906***
E	12.59±0.7279	13.16±0.7428	13.57±0.5412*	14.05±0.4722***	14.89±0.1179***
F	12.43±1.491	13.02±1.409	13.43±1.354	14.21±1.068	15.05±0.7689**

Values are given as Mean± S.E.M (n=6); ***p<0.001; **p<0.01;*p<0.05 compared with control

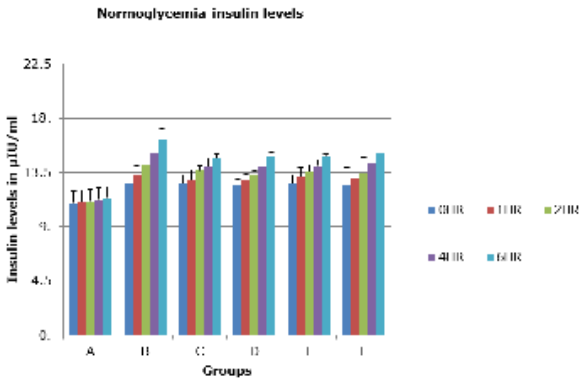
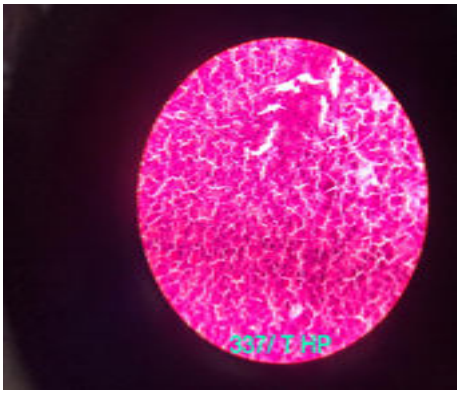


FIGURE 5 Normoglyceamic studies(BIL)

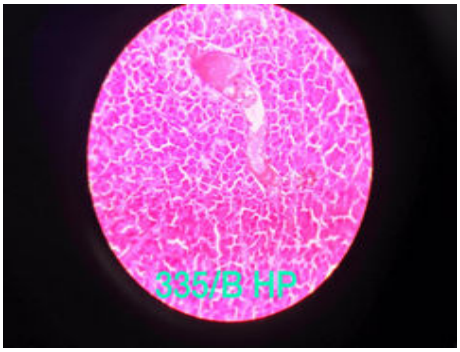
In normal rats the insulin levels were measured. Better response (p<0.001)was found in standard drug only from 2 hr. Drug at all doses showed gradual increase in insulin levels from 2 hrs but was not relevant response as that of standard drug

HISTOPATHOLOGICAL OBSERVATION:



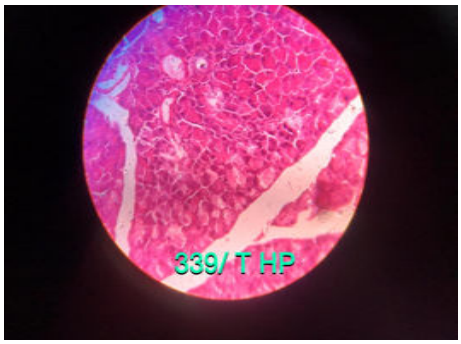
PANCREAS TRANVERSE SECTION OF CONTROL GROUP ANIMAL:337/T/19 Heamatoxylin and eosin stained section shows pancreatic tissue arranged in aacinar pattern with individual cells are round to oval with vesicular nucleus and abundant esinophilic cytoplasm. Occasional areas show islets cells with granular cytoplasm, few areas show moderate hemorrhage and foci of chronic inflammatory infiltrate.

Suggestive of normal pancreatic tissue

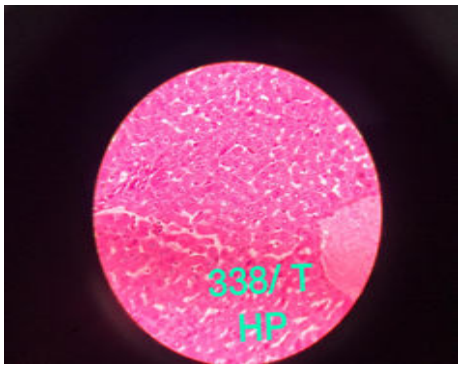


PANCREAS STANDARD DRUG GROUP ANIMAL SLIDE 335/B/19 Hematoxylin and eosin stained section shows pancreatic tissue with acini. Individual cells are oval to round with vesicular nucleus with granular cytoplasm .Most of the acini are displayed with moderate amount of haemorrhage and their ducts are congested. Few of the cells show fatty changes. Clustters of Islets of Langerhans are seen.

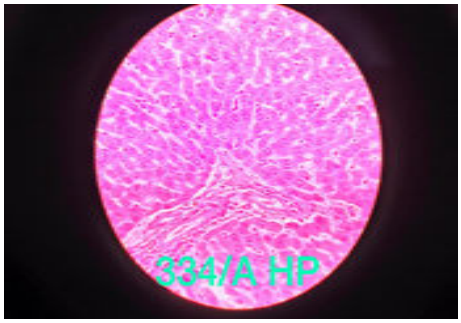
Features are s/o pancreatic tissue with moderate haemorrhage.



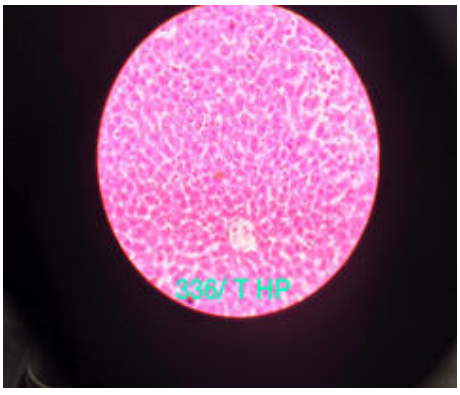
PANCREASE FROM TEST GROUP 339/T/19 Hematoxylin and eosin stained section shows pancreatic tissue arranged in lobules with the adjacent large amount of adipose tissue. The individual cells are round to oval with acinar pattern, **most showing granular cytoplasm** and some cells showing pale pink cytoplasm within the lobules. Occasional cells shows binucleated with pale pink cytoplasm. Adjacent to pancreatic tissue is the strip of adipose tissue along with lymphnode with mild changes. Pancreatic ducts – small and large ducts with minimal inflammatory cell infilterates and moderate amount of haemorrhage.



LIVER CONTROL GROUP 338/T/19 Hematoxylin and eosin stained section showing liver tissue arranged in lobules seperated by Fibrocollagenous strands. Each lobule composed of hepatocytes arranged closely in columns with a large central vein filled with blood elements, and peripheral portal triad. Most of portal tracts are collapsed and few shown mild inflammatory infilterate composed of lymphocytes and neutrophils. Some of the central veins are empty. Occasional hepatocytes show fatty changes.H/O suggestive of normal liver tissue with moderate amount of haemorrhage.



LIVER TS OF STANDARD DRUG TREATED ANIMAL 334/A/19 Hematoxylin and eosin stained section show liver tissue with congested blood vessels .Individual cells are arranged in lobules arranged around the central veins.Some are congested and some peripherally located portal triads show inflammatory cell collections. Some of the hepatic cells show degeneration, amidst few regenerated hepatocytes also seen. Moderate amount of fatty change seen with mild inflammatory cells.Significant amount of haemorrhage surrounding the liver tissue noticed.Features suggestive of liver tissues with moderate haemorrhage.



LIVER TEST GROUP 336/T/19 Heamotoxylin and eosin stained section shows liver tissue with normal hepatocytes arranged in lobules Amidst the binucleated cells seen.Mild congestion of central vein and mild fibrosis and with inflammatory infiltration of the portal triad with haemorrhage. Features suggestive of Hepatic tissue with reactive changes.

DISCUSSION

In hypoglycaemic Studies or Normoglycemic Studies ,Insulin Levels - P<0.001was seen in standard drug group B accordingly D group & E group showed similar results of P<0.001 equivalent to standard group.Glucose Levels - Group C & Group F have reduced to P< 0.01 but standard has P< 0.001.Inference: This shows that the research drug might have increased insulin levels but it is ineffective insulin by research drugs in instant dosing in healthy non-diabetic animals.

In Short term studies reveals blood Insulin of Group B,C and F have P value < 0.001 on 24 hours study. Glucose Levels of Groups C,E and F have glucose levels equivalent to Group B i.e P<0.001. Inference: There is similar action of both research drugs and standard drug on blood insulin levels and glucose levels on diabetes induced animals with instant drug dose.

Long Term studies: Blood insulin levels of animals of Group C,E and F have increased in par with the standard drug Group B i.e P<0.001 on 14th day. Blood glucose had a marked reduction of glucose (P value 0.0001) of Group C,E and F is seen on 14th day compared to Standard drug in Group B(P=0.05). Inference:

Increase in blood insulin levels for Groups C,E and F is equivalent to standard drug i.e P=0.001 on 14th day. But the glucose level reduction was approximately 34% of standard drug. The research drug reduction of glucose level percentage in each group varied from Group C- 29.81%, Group D- 37.70, Group E- 42.3, Group F- 70.25%.Though the percentage varied in all test groups but the end result of the blood sugar level was always around the renal threshold for blood glucose i.e 180mg/dl. This shows the efficacy of the synergistic action of biological drugs that can be biologically more tailored in its action compared to synthetic drug.

Histopathological findings of pancreas and Liver of the animals treated with test drug has higher concentration of granular substances in the cytoplasm of the cells which is the sign of precursor of insulin.

CONCLUSION:

The results were statistically analyzed utilizing one way analysis ANOVA using computerized Graph pad Instat version 3.05 and were considered statistically significant when P<0.05. The study reveals that the drug has hypoglycaemic action and also increases the production of insulin thus the poly herbal medicine has anti-diabetic activity when compared with a standard anti diabetic drug in animals.

REFERENCES:

1. Awad M. Ahmed .saudi med journal2001 Vol 23 (4):373-378
2. WHO Diabetes Over view. Web site: who.int/ health-topics/diabetes #tab=tab_1.
3. Sarwar N, Gao P, Seshasai S R, Gobin R, Kaptuge S, Di Agelantio et al Diabetes Mellitus, fasting blood glucose concentration, and risk of vascular disease , a collaborative meta-analysis of 102 prospective studies. Emerging Risk Factors Collaboration. By.Lancet 2010 26,375.2215-2222).
4. WHO Website Diabetes 8th June 2020. Who.int/ news-room/factsheets/ detail/diabetes viewed on 6th Jan 2021.
5. A R Murthy, R H Singh-Concept of Prameha / Madhumeha contradictions and compromises.Ancient Science of life.,30 Jan 1987Volume IX No.2
6. Halliwell B, Gutteridge J (1989) Free radicals in biology and medicine. Clarendon Press, Oxford.
7. Tolman KG, Fonseca V, Dalpiaz A, Tan MH (2007) Spectrum of Liver Disease in Type 2 Diabetes and Management of Patients with Diabetes and Liver Disease. Diabetes care 30: 734-743.
8. Liu HR, Tang XY, Dai DZ, Dai Y (2008) Ethanol extracts of Rehmannia complex [Di Huang] containing no corni fructus improve early diabetic nephropathy by combining suppression on the ET-ROS axis with modulate hypoglycemic effect in rats. J Ethnopharmacol 118: 466-472.
9. Vestra M, Fioretto P (2003) Diabetic nephropathy: renal structural studies in type 1 and type 2 diabetic patients. Internatrional Congress Series 1253: 163-169.
10. Cameron NE, Gibson TM, Nangle MR, Cotter MA (2005) Inhibitors of advanced glycation end product formation and neurovascular dysfunction in experimental diabetes. Ann N Y Acad Sci 1043: 784-792.
11. Kalia K, Sharma S, Mistry K (2004) Non-enzymatic glycosylation of immunoglobulins in diabetic nephropathy. Clin Chim Acta 347: 169-76.
12. Jandeleit-Dahm KA, Lassila M, Allen TJ (2005) Advanced glycation end products in diabetes-associated atherosclerosis and renal disease: interventional studies. Ann N Y Acad Sci 1043: 759-766.
13. Ramesh B, Pugalendi KV (2006) Impact of umbelliferone [7-hydroxycoumarin] on hepatic marker enzymes in streptozotocin diabetic rats. Indian J Pharmacol 38: 209-210.
14. Vagbhatta Ashtanga Hridaya Nidanasthana Chapter 11 Shloka 21.
15. Barnett DM, Krall LP. In: The History of Diabetes. 14th ed. Joslin's Diabetes Mellitus, editor. Boston, Massachusetts: Lippincott Williams & Wilkins; 2005. [Google Scholar]
16. Madhavakara , Madhava nidhana, 33 rd Chapter , 1 Sloka.
17. Vagbhatta Ashtanga Hridaya Nidanasthana , Chapter 10 Shloka 7.
18. Charka Samhitha Nidana Sthana Chapter 4 Shloka 5,8,24,36, Chikitsa Sthana chapter 6 Shloka4.
19. Charka Samhitha Chikitsa Sthana chapter 11 Shloka 3.
20. Causes of vision loss world wide, 1990-2010 Asystemic Analysis. Bourne RR1Stevens GA, white RA, Smith JL, Flaxman SR, Price H et al, Lancet Global Health.
21. Mutalik S, Sulochana B, Chetana M, Udupa N, Uma Devi P (2003) Preliminary studies on acute and subacute toxicity of an antidiabetic herbal preparation, Dianex. Indian J Exp Biol 41: 316-320.
22. Halberstein RA (2005) Medicinal plants: historical and cross-cultural usage patterns. Ann Epidemiol 15: 686-699.
23. Sherifali D, Nerenberg K, Pullenayegum E, Cheng JE, Gerstein HC. The effect of oral anti-diabetic agents on A1C Levels: A systematic review and meta-analysis. Diabetes Care 2010;33:1859-64.)
24. J. K. Grover, S. Yadav, and V. Vats, "Medicinal plants of India with anti-diabetic potential," Journal of Ethnopharmacology, vol. 81, no. 1, pp. 81–100, 2002.View at: Publisher Site | Google Scholar].
25. P. A. Babu, G. Suneetha, R. Boddepalli et al., "A database of 389 medicinal plants for diabetes," *Bioinformation*, vol. 1, no. 4, pp. 130–131, 2006.View at: Publisher Site | Google Scholar].
26. Hongxiang Hui*1,3, George Tang2 and Vay Liang W Go3, "Hypoglycemic herbs and their action mechanisms" 12 June 2009 Chinese Medicine 2009, 4:11 doi:10.1186/1749-8546-4-11 Received:
27. J Assoc Physicians India. 2005 Jan;53:34-8. Epidemiology of diabetes in India--three decades of research Ramachandran A1 .
28. Said HM., 1996.
29. Philipson JD., 1989.
31. Waer HF, Helmy SA (2012) Cytological and Histochemical Studies in Rat Liver and Pancreas during Progression of Streptozotocin Induced Diabetes and Possible Protection of Certain Natural Antioxidants. J Nutr Food Sci 2:165. doi:10.4172/2155-9600.1000165.
31. Buger G T, Miller CL, Animal care and facilities: In principles and methods of toxicology, Wallace Hayes A, NewYork, Raven press Ltd, 2, 1989, pp 527-531.
32. Veerappan, S. Miyajakib, M. Kadarkaraisamyc et al., Acute and sub-acute toxicity studies of *Aegle marmalose* corr., an Indian medicinal plant, Phytomedicine, 14, 2007, pp 209-215.
33. Williamson E.M, Okpoko D.T, Evans F.J, Pharmacological Methods in Phytotherapy Research, 2, 1996, pp 155-167.

34. Etuk, E.U, Animal models for studying diabetes mellitus, (2010), Agricultural and Biology, J.N.Am1(2):pp 130-134.
35. Trivedi N A.Mazumdar B. Bhatti J D and Hemavathi K.G.Effects of Shilagit on blood glucose and lipid profile in Alloxan induced diabetic rats, Indian J Phamacology 36,2004,pp373-376.
36. Mitra SK, Gopumadhavan S, Muualidhar TS, Anturlikar SD, Sujatha MB (1996) Effect of a herbo-mineral preparation D-400 in streptozotoci-induced diabetic rats. J Ethno-pharmacol 54: 41-46.
37. Trinder P, Determination of blood glucose using 4-amino phenazone as oxygen acceptor, J. Clin. Pathol, 1969, pp 22, 246
38. Jamshidzadeh A, Baghban M, Azarpira N, Mohammadi Bardbori A, Niknahad H (2008) Effects of tomato extract on oxidative stress induced toxicity in different organs of rats. Food Chem Toxicol 46: 3612-3615.
39. Luna, Luna L G, Manual of histology and staining methods of armed forces institute of pathology, 3rd ed, New York, McGraw Hill Book Co, 1986, pp 1-31.