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Preclinical antidiabetic efficacy of polyherbal formulation (PHF-3) for the treatment of madhumeha

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ABSTRACT: Madhumeha is a disease known since ancient times to the mankind.In present eraMadhumeha is increasing among the general population due to life style modifications, stress & strain, change in dietary habits and industrialization. On the basis of its symptomatology Madhumeha can be correlated to the features of Diabetes Mellitus. The antidiabetic activity of the individual plant parts is well known but combined effects are unclear. The aim of the present study is to formulate a polyherbal formulation and evaluate its antidiabetic potential in animals. The concept of polyherbal formulation get from Charak Samhita (6th chapter), an ayurvedic literature. The polyherbal formulation (PHF - 3) was formulated using the aquaous extract of Rhizome of Haridra (Curcuma longa), Stembark of Daruharidra (Berberisaristata), Fruit of vidanga (Embelia ribs).The antidiabetic activity of PHF-3 was evaluated by different scientific parameters after performing the pharmacognostic study, phytochemical study, TLC, UV-spectrophotometry analysis, HPLC and HPTLC analysis experimental study to confirm its antidiabetic efficacy. HPLC analysis showed three main peak due to isolation of three main compounds of PHF-3 which eluted on 280nm. The acute toxicity studies of the PHF – 3 didn't show any toxic symptoms in doses up to 1000 mg/kg over 14 days.Oral glucose tolerance Test (OGTT)was performed on wister ratthose who are fasting for 18hours with glucose at a dose 2gm per kg body wt. Blood glucose level was monitored at 0 min(just prior glucose administration), 30, 60, and 90 min intervals and reported as the average glucose level of each group.The oral antidiabetic activity of the polyherbal formulation (500and 700mg/kg)was screened against alloxan(150mg/kg body wtip) induced diabetes mellites in rats. PHF – 3 was administered for 14 consecutive days. Blood sample was drawn by one touch glucometer on 3rd day, 7th day and 14th day.Polyherbal formulation showed significant antidiabetic activity at 500 and 700 mg respectively and this effect was comparable with that of Glibenclamide.

Key words: Madhumeha, polyherbal formulation,Haridra (Curcuma longa), Stembark of Daruharidra (Berberisaristata), Fruit of vidanga (Embelia ribs).

I. INTRODUCTION:

Madhumehahas been a disease of great concernsince vedic period. On the basis of its symptomatology Madhumeha can be correlated to the features of Diabetes mellitus.Diabetes Mellitus is a clinical syndrome characterized by hyperglycaemia due to absolute or relative deficiency of insulin. The decrease secretion of insulin affects the metabolism of carbohydrate, protein and fat, can cause significant disturbance of water and electrolyte homoeostasis.Diabetes has been described as an epidemic. Current estimates suggest that the prevalence of type 2 diabetes worldwide is set to increase from its present level of 150 million to 250 million by the end of the decade and 300 million by 2025.Ayurveda because of its holistic approach not only aims to achieve strict glycaemic control but also treat root cause of the disease. Our ancestors mentioned lots of medicine for management for Madhumeha without hazardous side effects.MaharshiCharaka in Charaksamhitachikitsasthana 6/27 has mentioned lots of pramehahara yoga(polyherbal formulation) for management of madhumeha.Among the yoga one was selected for my scientific study.Compare to the single herb, the polyherbal formulation as better and extended therapeutic potential. Hence, the presence study was planned to formulate and standardised a polyherbal formulation using plants having known antidiabetic activity and evaluate its therapeutic effects in animals.

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II. MATERIALS AND METHOD:

Plant materials:

Rhizome of Haridra (Curcuma longa), Stembark of Daruharidra (Berberisaristata), Fruit of vidanga (Embelia ribs). were collected from a reputed local supplier & authenticated by the Dept. of Dravyagunaof IPGAER at SVSP,KOLKATA.

Chemicals:

The chemicals were collected from the reputed licenced supplier of the institution.

Chemicals were used for preparation of extraction of research drug are Petroleum ether, Chloroform, Acetone,Ethyle acetate &Distilled water.Chemicals were used for phytochemical study are Mayers reagent, Dragendorff's reagent, Fehling solution, Ferric chloride solution etc.Chemicals were used for the experimental study are glucose, Alloxan monohydrate and Glibenclamide.

Animals :

The animals for experimental purpose were collected from an authorized licenced breeder and legal authorization was taken from **CPCSEA**.**Albino mice** were taken for acute toxicity test. **Wister rats** of either sex were chosen for the experiment of oral glucose tolerance test model and alloxan induced diabetic model.

Preparation of research drug :

The shadow,air dried Rhizome of Haridra (Curcuma longa)Stembark of Daruharidra (Berberisaristata) Fruit of vidanga (Embelia ribs)were powderedand extractedwith distilled water using soxhlet apparatus from nonpolar to polar solvent.The extract were evaporated to dryness and stored at 4 degreeCelcius until use.

Pharmacognostic study:

Pharmacognostic study was performed in the laboratory of Dravyagunavijnan in the institute of I.P.G.A.E.&R following the standard guidelines of AyurvedicPharmacopeia.Mainly two types of study were done through this study. In macroscopic study individual component of PHF-3 were analysed. In microscopic study powder microscopy of PHF-3 were done individually and combined.

Physiochemical study:

Physiochemical parameters of raw materials were determined as per the guidelines of the WHO, which includes following:

Study of physical parameters: Determination of moisture value, Determination of PH value, Determination of ash value, Determination of extractive values in different solvent system.

Study of chemical parameters: One gram of prepared extract was dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1%w/v and tested for the presence of carbohydrates, proteins, saponins, steroids, flavonoids, tannins, glycosides, triterpenoids etc., Chromatographic analysis TLC (Thin layer chromatography) HPLC (High performance liquid chromatography) HPTLC (High performance Thin layer chromatography), Spectroscopy analysis – By UV- visible spectroscope.

pH value: pH of 1% solution was determined by using a digital pH meter.

Ash value and extractive value: Total Ash, water soluble Ash, acid soluble Ash and extractive values were determined as the procedure described elsewhere.

Moisture content: moisture content was determined by using moisture containing apparatus.

TLC (Thin layer chromatography): TLC of hydroalcoholic extract was done on silica gel coated plate on following solvent system(Toluene:Ethyleacetate:Glacial acetic acid:Formic acid (7:2:0.5:0.5)) and observation were done under the UV chamber,invisible light,254 nm(short) and 356(long) UV wave length.spots were noed.Rf value of each spot were calculated.

HPLC(High performance liquid chromatography):The chromatogram of HPLC analysis showed three main peak due to isolation of three main compounds of PHF-3 which eluted on 280 nm.The retention time of first ,second and third peak were 5.739 min, 12.149 min and 12.910 min respectively.

UV-VISIBLE SPECTROCSOPY:UV Spectophotogram of methanolic extract of (PHF-3) was done using wave length 190-900 nm.The first pick came on 422.50 nm where absorbance was 1.462,the second pick came on 292.50 where absorbance 1.428,third pick came on 211.00 where absorbance 2.090.

HPTLC (High performance thin layer chromatography):The HPTLC of methanolic extract showed separation of 3 compound on the basis of Rf value in the solvent system of (Toluene:Ethyleacetate:Glacial acetic acid:Formic acid)- (7:2:0.5:0.5). Rf value of first ,second and third peak were 0.00,0.07,0.22 respectively.

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4.Experimental Study:

The experimental studies were done on the Albino mice& Wister rat in the animal house under the Department of Dravyagunavijnan (RegistrationNo- 1180/AC/ CPCSEA dated 27.03.2008) in I.P.G.A.E.R. atS.V.S.P.according to the guidelines of CPCSEA after the approval of IAEC. Throughout the experimental study following test were done:

Acute Toxicity Test

The acute toxicity of the research drug was done by following the guidelines of OECD 423 to find out the toxic effect and LD-50(Lethal dose) of the drug on Albino mice having weight (20-25) gm.Mice were divided into 5 groups having three animals in each group. The research drug was given to the animal orally in the different dosages-100mg/kg, 300 mg/kg, 500 mg/kg, 700 mg/kg & 1000 mg/kg body wt. The animals were fasted for overnight , after that the drug was administered orally to each animal as per their body weight and the following symptoms such as salivation, lacrimation, convulsion andother toxic effect was observed at a interval of 1hr, 2hrs, 4hrs, 8hrs, 12hrs & 24hrs observation was done upto 14 days for 24 hours.Signs and symptoms were recorded.

Oral glucose tolerance Test (OGTT):

OGTT was performed to evaluate the peripheral glucose utilization. Twenty four wisterrats (150-200) gm were randomly assigned to 4 groups consisting of six animals. They will be fasted for about 18 hrs. Glucometer was used to estimate their fasting blood sugar level.

After that the animals were administered orally as follows:

Group 1- distilled water served as control group
Group 2-Glibenclamide(600µg/kg body weight)
Group 3-research drug PHF- 3(500mg/kg body weight)
Group 4-research drug PHF- 3(700mg/kg body weight)

After 30 min, each animal was administered orally with glucose at a dose of 2gm/kgbwt. Blood glucose level was monitored at 0 min(just prior glucose administration), 30, 60, and 90 min. intervals and reported as the average glucose level of each group.

Evaluation of Antidiabetic Activity Of (PHF-3) In Alloxan-Induced Diabetic Model:

Atfirst alloxan monohydrate was weighed for each rat according to body weight(150 mg/kg bwt). Diabetes was induced inovernight fasted rat by intraperitoneal (ip) injection of alloxan monohydrate dissolved in normal saline . After 72 hrs, blood was withdrawn for blood glucose estimation monitored with one touch glucometer. The animals with blood glucose level ≥ 250 mg/dl was considered diabetic and included in the experiment.

The diabetic animals were randomly distributed into four groups of six animals each. Blood sample was drawn by one touch glucometer on 3rd day, 7th day and 14thday.Animals were administered orally as follows:

Group 1: distilled water served as control group.
Group 2: Glibenclamide 600µg /kg bwt
Group 3: (PHF- 3) at a dose of 500mg/kg body weight.
Group 4: (PHF- 3) at a dose of 700mg/kg body weight

III. RESULT:

Physiochemical analysis:

Showing Extractive value of PHF-3 in different solvent system:

Solvents (According to polarity)	Extractive - value	Colour
Petroleum Ether	1.44%	Yellowish
Chloroform	0.09%	yellowish brown
Ethylacetate	0.61%	brown
Acetone	0.76%	Dark yellowish Brown
Ethyl Alcohol	0.74%	Dark Brown
Water	1.48%	Coffee brown

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Showing the result of physiochemical parameter of research drug(PHF-3):

Physiochemical parameter	Observation
Moisture content of research drug PHF-3	**2.1%**
Total ash value of research drug PHF-3	**7.9%**
Water soluble ash value of research drug PHF-3	**6.7%**
Acid insoluble ash value of research drug PHF-3	**1.2%**
PH value research drug PHF-3	**4.2**

Showing the chemical constituents of research drug(PHF3):

Test or reagent	Ethyl Alcohol extract	Water extract
Alkaloid (Mayers Reagent)	+	+
DragendroffReagent	+	+
Carbohydrate Fehling reagent	+	+
Saponin	-	-
Phenolic compoundand Tannin Ferric chlorideSolution	+	+
Protein and amino acid	+	+
Flavonoids	+	+
Glycosides	+	+
Triterpenoids and sterol	+	+

Chromatographic analysis
Thin layer chromatography:
Rfvalue of aqueous& alcoholic extract (PHF-3) of each spot were calculated which is given below the following table

Spot	1st	2nd	3rd
Rf of aquous extract	0.33	0.41	0.48
Rf of alcoholic extract	0.37	0.46	0.49

UV Spectroscopy analysis:
The UV visible spectrophotometric graph

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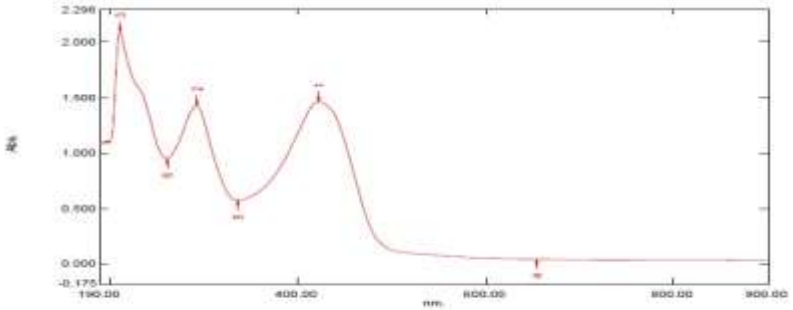
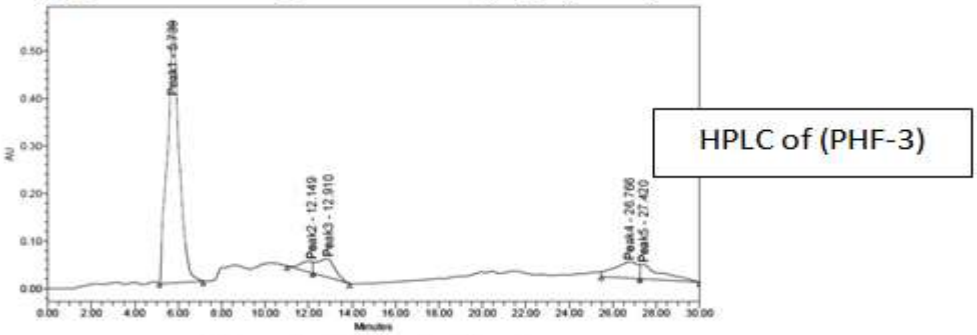


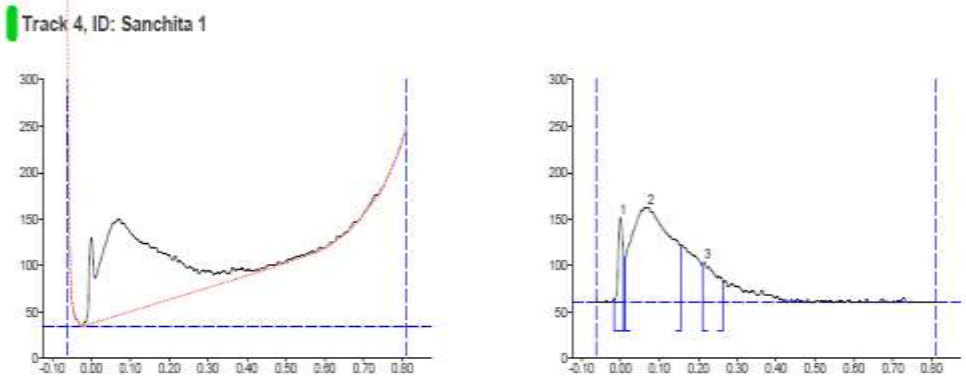
No.	P/V	Wavelength	Abs.	Description
1		422.50	1.462	
2		282.50	1.428	
3		211.00	2.096	
4		654.00	0.041	
5		336.50	0.575	
6		261.00	0.953	

High performance liquid chromatography analysis:



	Peak Name	RT	Area	% Area	Height
1	Peak1	5.739	20358280	72.49	553212
2	Peak2	12.149	850101	3.03	23955
3	Peak3	12.910	2190038	7.81	38954
4	Peak4	26.766	2598218	9.25	34333
5	Peak5	27.420	2084776	7.42	30427

High Performance Thin Layer Chromatographic analysis:



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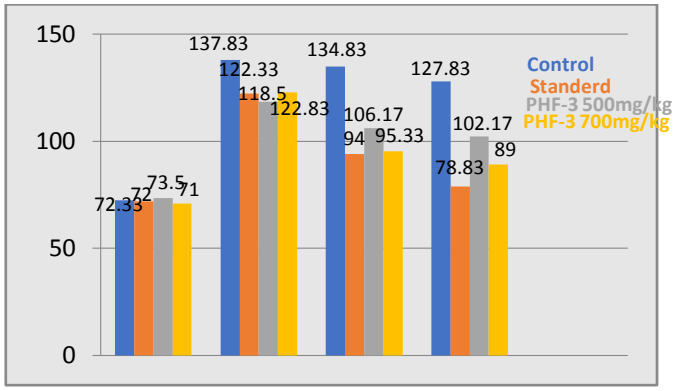
Experiential study:



Blood Glucose level in different group of oral glucose tolerance test

At 30 minute after glucose administration,the peak of blood glucose level increased rapidly from the fasting value in all group,and then subsequently decreased at 60 min 90 min except contol group and.In 60 min and 90 min research drug at dose of700mg/kg body weight exhibited remarkable blood glucose lowering effect which was very close to the standard drug.But in 90 min, standard drug exhibited more effective result than 700mg/kg of PHF-3 body weight.

Group	0 min Mean±SEM	30min Mean±SEM	60 min Mean±SEM	90 min Mean±SEM
Control	72.33±0.988	137.83±2.006	134.83±1.57	127.83±5.42
Standard	72±0.96	122.33±1.90	94±1.15***	78.83±1.49***
PHF-3 (500 mg/kg)	73.5±1.25	118.5±2.44	106.17±1.22**	102.17±2.58*
PHF-3 (700 mg/kg)	71±1.06	122.83±1.81	95.33±1.85***	89±1.65**

Oral glucose tolerance test in rats showing in different time period:
The result was expressed inMean±SEM ,where n=6,*p<0.01,p<0.001 as compared with control group (Annova test)**

Observation of alloxan induced diabetes in rat

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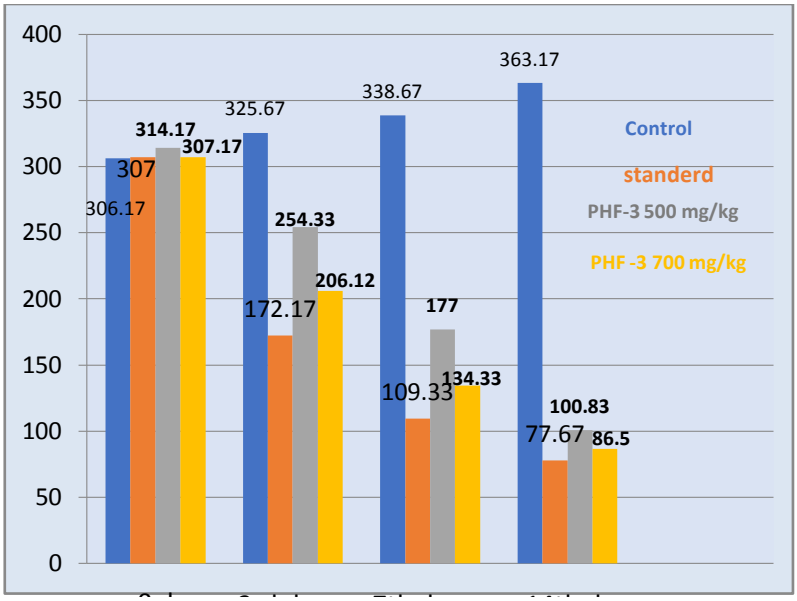
Group	0 day Mean±SEM	3rd day Mean±SEM	7th day Mean±SEM	14thdayMean±SEM
Control	306.17±32.84	325.67±30.40	338.67±30.57	363.17±29.95
Standard	307±47.56	172.17±19.52	109.33±13.89	77.67±8.79**
PHF-3 (500 mg/kg)	314.17±15.93	254.33±14.42	177±17.31	100.83±2.87*
PHF-3 (700 mg/kg)	307.17±15.93	206.12±22.67	134.33±3.01	86.5±2.95*

The result was expressed inMean±SEM ,where n=6,*p<0.01,p<0.001 as compared with control group (Annova test)**

Observation of alloxan induced diabetes in rats



Hypoglycaemic effect in different group of alloxan induced diabetic model

In 14th day blood glucose level significantly decreased in standard group, PHF-3 500mg/kg & PHF-3 700mg/kg but high increased in control group.PHF-3 700mg/kg group showed more hypoglycaemic activity than PHF-3 500mg/kg. Percentage of inhibition of blood glucose level in 14th day in standard group ,PHF-3 500mg/kg & PHF-3 700mg/kg 74.70% ,67.90% ,71.83%Respectively.

IV. DISCUSSION:

The present era is full of chaos, stress & strain due to life stylemodifications, change in dietary habits, urbanization and industrialization. This has lead in the upsurge of many diseases and one of them is Madhumeha. Though Madhumeha is a disease known since ancient times to the mankind, its upsurge is quiet alarming. On the basis of its symptomatology Madhumeha can be correlated to the features of Diabetes mellitus type-2. Diabetes mellitus is a metabolic disorder of carbohydrate, fat, & protein characterized by hyperglycaemia with or without glycosuria It is one

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of the leading causes of morbidity and mortality. Recent survey conducted by World Health Organization (W.H.O.) has revealed that India is having highest number of diabetics in the world.In this condition safe,cost effective, antidiabetic Ayurvedic drug is required for management of Diabetes mellitus without hazardous side effects. In Charak Samhita,chapter 6thMaharshiCharak mentioned pramehahara yoga(polyherbal formulation) for management of Madhumeha having the equal amount of ingredient such as Rhizome of Haridra (Curcuma longa Linn.), Stem berk of Daruharidra(Berberisaristata Dc.),Fruit of Vidanga(EmbeliaribesBurm) named a formulation PHF-3.The antidiabetic activity of PHF-3 was evaluated by different scientific parameters after performing the pharmacognostic study,phytochemical study,experimental study to confirm its antidiabetic efficacy.The TLC of aqueous extract showed presence of three compounds in the solvent system of Toluene:Ethyleacetate:Glacial acetic acid:Formic acid in ratio of (7:2:0.5:0.5).This study has been confirmed by UV-spectrophotomety analysis,HPLC and HPTLC analysis on the basis of peaks. HPLC analysis showed three main peak due to isolation of three main compounds of PHF-3 which eluted on 280 nm. The retention time of first,second and third peak were 5.739 min, 12.149 min and 12.910 min respectively.

After standardization of PHF-3,It has been used for experimental study which includes acute toxicity,oral glucose tolerance test and alloxan induced diabetic model.There was no such toxicity or no mortality found upto the dose of 1000mg/kg body weight in acute toxicity test following the OECD guideline. In oral glucose tolerance test at 30 min after oral glucose administration, the blood glucose level increased rapidly from the fasting value in all groups and then subsequently decreased at 60 min and 90 min in all groups except control group. The test drug PHF-3 at the dose of 700mg/kg body weight ($p<0.01$) and (PHF-3) 500mg/kg($p<0.05$) body weight both were exhibited remarkable significantblood glucose lowering effectin 60 min. In 90 min,the result of (PHF-3) at 500mg/kg body weight is significant($p<0.01$) & (PHF-3) 700mg/kg body weight exhibited highly significant antidiabetic and sustained effect which was very close to the standard drug ($p<0.001$).So the maximum hypoglycaemic effect of PHF-3 has been observed at 90 min Comparing the control and standard group.This hypoglycaemic activity may be due to presence of chemical constituents like Curcumin,Berberine and Embelin in (PHF-3). Berberine lower the blood glucose level through inhibition of α-glucosidase,activation of AMPK pathway and stimulation of glycolysis. Embelin regulate insulin mediated glucose uptake in epididymal adipose tissue through translocation and activation of GLUT4.On other hand inhibitory action of curcumin on HPA(Human Pancreatic Amylase) causes reduction in starch hydrolysis leading to lower glucose levels.

In alloxan induced diabetic model it has been observed that hypoglycaemic activity were found in both doses at 500mg/kg body weight & 700mg/kg body weight of (PHF-3)and it was significant at $p<0.01$ in Comparison with the control group.In 14th day the percentage of inhibition of blood glucose in (PHF-3) 500mg/kg body weight and PHF-3 700mg/kg body weight and standard group were 67.90%,71.83%,74.70% respectively. This hypoglycaemic activity due to presence of alkaloids like Curcumin,Berberine and Embelin present in (PHF-3).Because it has been already established that both curcumin and embelin increase insulin secretion,regulate insulin resistance, alter β-cell dysfunction.(42,43,44) On other hand Berberine improve insulin secretion via resuscitating exhausted islets.

So cumulated all above mentioned research studies showed the research drug (PHF-3) is a safe,non - toxic, antidiabetic, quality control Ayurvedic formulation and proofed scientifically its madhumehahar (antidiabetic activity) mentioned in the Chark Samhita which can be used in a large number of human subjects in future.

V. CONCLUSION:

Through this study,the antidiabetic activity of research drug namely (PHF-3) was evaluated by following standard guideline of drug development & various scientific parameter after performing the pharmacognostic study,phytochemical study,toxicological screening,animal experimental study.In pharmacognostic study, Macroscopic structure & powder microscopy was observed.In phytochemicals study alkaloid,phenolic compound,tannin,flavonoid, glycoside,triterpenoids were found in (PHF-3).TheTLC of aqueous extract showed presence of three compounds which was confirmed by UV-spectrophotometry analysis,HPLC and HPTLC analysis on the basis of peaks.In experimental study,there was no such toxicity or no morality found up to the dose of 1000mg/kg body weight in acute toxicity test. The drug (PHF-3) proved its sustained hypoglycaemic

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activity in oral glucose tolerance test and alloxan induced diabetic model due to presence of alkaloids, phenolic compound & Glycosides. So considering the cumulative effect of the drug (PHF-3), it can be concluded that (PHF-3) the drug has been significantly evaluated and proved its antidiabetic activity following standard parameter.

Ethical clearance

Taken from CPCSEA(Committee for the Purpose of Control and Supervision of Experiments on Animals)

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