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EVALUATION OF ANTI DIABETIC ACTIVITY AND ITS COMPLICATIONS OF HYDRO -ALCOHOLIC POLYHERBAL MIXTURES IN ALBINO WISTAR RATS

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ABSTRACT

In the current investigation, diabetic wistar rats were induced with nicotinamide-streptozotocin, and the hydroalcoholic extract of polyherbal mixtures combinations of *Ocimum tenuiflorum, syzygium cumini* and *Aegel Marmelos* was utilised to assess its antihyperglycemic and anti-neuropathic activities. To test anti-diabetic activity, doses were given like standard– Metformine, PHM-1 with 200 mg/kg (low dose), PHM-2 with 400 mg/kg (medium dose) and PHM-3 with 800 mg/kg (high dose). After the administration for 21 days the PHM -3(low dose) was more accurate to treat diabetes and multiple parameters including triglyceride (TG), total cholesterol (TC), SGOT, SGPT, serum creatinine, serum bun, and uric acid are seen. To compare with other treatment groups, metformin is used. Eddy's hot plate and hot water immersion method and cold allodynia method with the doses of polyherbal mixtures combination of *Ocimum tenuiflorum, syzygium cumini* and *Aegel Marmelos* were given like standard-Pregablin, PHM-1 with 200 mg/kg (low dose) and PHM-2 with 800 mg/kg (high dose) is used to assess neuropathic action. Using the sciatic nerve ligation method, eddy's hot plate method, hot water immersion method and cold allodynia, neuropathic activity is assessed by the animals pain tolerance and pain frequency after administrating the drug for 21 days the PHM-1 (High dose) shows the better analgesic activity on rats when compared to PHM2 .The Histopathological examination confirmed the antihyperglycemic and analgesic effect of PHM. Among the three doses PHM3 the high dose is statistically significant with ***p<0.001 when compared to Disease control. And comparing PHM1

*p<0.05 and PHM2 **p<0.01 with disease control, where as in diabetic neuropathy, Among the two doses PHM3 is statistically significant with ***p<0.001, when comparing with disease control.

Keywords: Diabetic neuropathy, diabetes, polyherbal mixtures, tail flick method, Eddy's hot plate, cold allodynia method, Sciatic nerve ligation

INTRODUCTION:

Diabetes is a chronic illness characterised by raised glucose levels and improper protein and fat metabolism. Blood sugar levels were elevated because the pancreas was either not creating enough insulin or the cells were unable to absorb the insulin that was being generated **[1]**. Insulin encourages adipose and skeletal muscle cells to take up additional glucose by bringing the glucose transporter GLUT4 to the cell surface, in addition to instructing the liver to convert surplus glucose into glycogen for storage. This helps to get blood glucose levels back to normal. When blood glucose levels are low, the pancreatic cells become stimulated and release glucagon. Glucagon instructs the liver to release stored glucose into the circulation in order to attain stability **[2]**.

The two classifications of diabetes mellitus are insulin-dependent diabetes mellitus (Type I) and non-insulin-dependent diabetes mellitus (Type II). Type I diabetes is an autoimmune disease characterised by a localised inflammatory response in and around the islets that is followed by the selective death of insulin-secreting cells. This is in contrast to Type II diabetes, which is characterised by peripheral insulin resistance and impairment. There are three distinct types of diabetes, and they are as follows: Type 1 diabetes mellitus, commonly known as insulin-dependent diabetes mellitus, is a condition in which the body is unable to produce insulin, necessitating the use of an insulin pump or injections of the drug **[3]**. An alternative name for this is "juvenile diabetes." Insulin resistance is a condition where cells use insulin incorrectly. There are some complications in diabetes they are 1.diabetic wound healing, 2. Diabetic nephropathy, 3.diabetic retinopathy, 4.Diabetic-neuropathy **[4]**.

Diabetic wound healing: A wound is defined as a loss of structural and functional skin integrity at the affected site. Trauma initiates the biological process of wound healing, which is frequently completed by the formation of a scar. Human wound healing has various distinct elements that are dependent on physiology, age, sex, illness condition, and so on. Diabetes mellitus is one such illness condition that will result in various consequences, the most common and damaging of which are diabetic wounds **[5]**.

Diabetic nephropathy: diabetic nephropathy is defined by a steady increase in urine

albumin excretion, followed by an increase in blood pressure, which leads to decreased glomerular filtration and, eventually, end-stage renal failure. Diabetes nephropathy is now the most frequent cause of end-stage renal failure in the world, and it's also a separate risk factor for cardiovascular disease. In many countries, the majority of diabetic patients starting renal replacement therapy have type 2 diabetes rather than type 1. As a result, this study will involve both type 1 and type 2 diabetic nephropathy **[6]**.

Diabetic retinopathy: Diabetic retinopathy is one of the most prevalent diabetes-related squeal, with a global prevalence of approximately 1%. Sinclair and Schwartz Diabetic Retinopathy A 382 million Inflammatory, Neuro-Vascular Complication. In the United States, one-third of diabetic people over the age of 40 have diabetic retinopathy, with one in every six having visual loss. Retinal ischemia, neovascularization, altered retinal permeability, and macular edoema are all caused by changes in the microvasculature. Diabetic retinopathy is the leading cause of blindness in those who are still working **[7]**.

Diabetic neuropathy: Diabetic neuropathy is a loss of sensory function that begins in the lower extremities and causes severe morbidity and suffering. Diabetic neuropathy affects as least 50% of people with diabetes over the course of their lives. In individuals with type 1 diabetes, glucose management significantly slows the evolution of diabetic neuropathy, while the effects are less pronounced in those with type 2 diabetes. These discoveries have prompted additional research into the origin of diabetic neuropathy, as well as new 2017 guidelines on techniques to preventing and treating this illness that are tailored to each type of diabetes **[8]**.

In order to accomplish the real results of particular research goals, the need for the unique and particular model for diabetic complications has increased in recent years. Over the past few decades, numerous animal models of diabetic neuropathy have been created, each taking a different approach. However, because of their numerous drawbacks and restrictions, the most of them did not enjoy much popularity. A severe side effect of diabetes and the main reason for foot exclusion is peripheral diabetic neuropathy (PDN). Clinical signs of PDN include higher vibration and temperature thresholds that eventually lead to sensory loss, along with degeneration of all peripheral nerve fibre types **[9]**.

Clinical efforts have mostly concentrated on obtaining an effective management of blood glucose levels because there are no medications to prevent or treat DN. The vast majority of treatments for DN are unsuccessful and have detrimental side effects, which greatly reduce patient quality of life. The fundamental structural and

functional changes that occur when DN develops must be identified in order to develop more effective treatments. This study's main goal was to explain how DN started and progressed in this T2DM model. In order to accomplish this, we looked at the histology, sensory loss, and electrophysiological parameters of diabetic mice in the early, middle, and late stages of the illness. This work serves as a crucial beginning point for the creation of novel therapeutic trials for DN and for the determination of an appropriate time frame for the successful application of these possible treatments **[10]**.

Plant Profile

***Ocimum tenuiflorum**

Ocimum tenuiflorum, commonly known as holy basil, Tulsi or tulasi,is an aromatic perennial plant in the family Lamiaceae. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the south, Tulsi is cultivated for religious and traditional medicine purposes, and also for its essential oil. It is widely used as a herbal tea, commonly used in Ayurveda, and has a place within the vaishnava tradition of Hinduism in which devotees perform worship involving holy basil plants or leaves. the chemical constituents that were present in ocimum were Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and β-caryophyllene and ocimus posses anti diabetic activity.[11]

***Syzygium cumini**

syzygium cumini , commonly known as Malabar plum, Java plum, black plum, Jamun or jambolan, is an evergreen tropical tree in the flowering plant family Myrtaceae, and favoured for its fruit, timber, and ornamental value. It is native to the Indian Subcontinent, adjoining regions of Southeast Asia, including Myanmar, Sri Lanka, and the Andaman Islands. It can reach heights of up to 30 metres (98 ft) and can live more than 100 years. A rapidly growing plant, it is considered an invasive species in many world regions.The plant contains a variety of chemicals, including isoquercetin, kaemferol, ellagic acid, glucoside, and anthocyanins. The alkaloid, jamboline, and glycoside jambolin or antimellin, which prevents the diastatic conversion of starch into sugar, are thought to be present in the seeds **[12]**. Mycaminose,is the compound which has the anti-diabetic activity which is extracted from the seeds of the *syzygium cumini* **[13]**.

***Aegel marmelos:**

Aegle marmelos, is a deciduos shrub or small to medium-sized tree, up to 13 metres (43 feet) tall with slender drooping branches and rather open, irregular crown The leaf is trifoliate, alternate, each leaflet 5–14 cm, ovate with tapering or pointed tip and rounded base, untoothed or with shallow

rounded teeth. Young leaves are pale green or pinkish, finely hairy while mature leaves are dark green and completely smooth. Each leaf has 4–12 pairs of side veins which are joined at the margin. Bael contains bioactive substances like coumarin, xanthotoxol, imperatorin, aegeline, and marmeline in its fruits, bark, leaves, seeds, and roots. The anti-diabetic, anti-cancer, anti-fertility, anti-microbial, immunogenic, and insecticidal properties of these substances are also possible **[14]**. the ripen and dry fruits of *Aegle marmelos* consists of analgesic activity. Carvacrol was the constituent which was responsible for analgesic activity **[15]**.

MATERIALS AND METHODS:

Plantcollection and authentication: Early leaf twigs of 1.*Ocimum sanctum* (tulasi), 2.*Syzygium tenue* (neredu) and 3. Aegle *Marmelos* (bilwa patra) were collected from local areas of Visakhapatnam and authenticated by Prof. S.B. Padal, Taxonomist Andhra University Visakhapatnam, dried and powdered, then the powder was kept for cold maceration for 48 hours and filtered by vaccum filteration, then distilation was kept for 4 hours after that the obtained semi solid samples was collected , and stored.

Chemicals: Streptozotocin and Nicotinamide were procured from sainadh chemicals, Visakhapatnam.

Animals: Healthy albino Wister rats of approximately same age group and weight of 150-200g were procured from sai agences, Hyderabad a registered breeder. Animals were housed at institutes animal house facility in polypropyline cages and maintained under standard conditions (12 h light/dark cycle at 22 + or -2^{O} C and 55 + or - 5% relative humidity) they were fed with standard pellet diet and water *ad libitium.* All the work is done with approval from the institutional animal ethics committee (IAEC/VIPT/2021/04) under strict compliance of committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines. (Reg. no. 2003/PO/RE/18/CPSEA).

Preparation of solutions: Test drugs and Metformin were weighed approximately according to the animal weights and dissolved in sterile water and administered orally for experimental purpose all the test and standard solutions were prepared freshly prior to the administration.

Determination of Acute Oral Toxicity: The acute oral toxicity of poly herbal formulation was carried out according to (OECD) organization of Economic Cooperation and development guideline-425 by using male albino Wister rats (150-200g) which were maintained under standard conditions. Animals were kept for 12 h fasting prior to the experiment, only water was given *ad libitium*. Test drug was

administered to all the animals upto2000 mg/kg by using oral feeding tube and all the animals were observed individually for the signs of toxicity for the time intervals of 2hrs up to 24 hrs, the toxicity was seen at 2000 mg/kg and the $1/10^{th}$ dose was taken as low dose for the study **[16]**.

Induction of Diabetes: Diabetes was induced for the animimals which were fasted for overnight (no food but have allowed free access to water) niotinamide was given to the animals prior 30 min to the STZ by intraperitoneal injection (i.p.) after that by single intraperitoneal injection (i.p.) which was freshly prepared streptozotocin (STZ) dissolved in citrate buffer (65mg/kg,body weight).hyperglycemia was confirmed by elevated glucose levels in plasma, determined after 72 hrs of streptozotocin injection .animals which were conformed with high blood glucose(200mg/dL) were used for the study **[17]**.

Grouping of Animals for anti-diabetic activity:

The diabetic animals were randomly divided into 6 groups with containing 6 animals in each group. All groups were given with STZ-nicotinamide except normal control group and treatment protocal is as follows

Group I - Normal control (saline treatment)

Group II - Disease control (STZ-Nicotinamide treatment) (i.p)

Group III - (STZ-Nicotinamide (i.p)+PHM - I,800 mg/kg (p.o.)body weight)

Group IV - (STZ-Nicotinamide (I.p)+PHM - II,400 mg/kg(p.o.) body weight)

Group V - (STZ-Nicotinamide (i.p.)+PHM - III,200 mg/kg(p.o.) body weight)

Group VI - Standard group (STZ-Nicotinamide (i.p)+Metformin, (200)mg/kg(p.o.) body weight) **[18]**

Grouping of animals for Neuropathic activity:

The diabetic animals were randomly divided into 5 groups with containing 6 animals in each group. all groups were given with STZ-nicotinamide except normal control group and treatment protocol is as follows

Group I - Normal control (saline treatment)

Group II - Disease control (STZ-Nicotinamide treatment) (i.p)

Group III - (STZ-Nicotinamide (i.p)+ PHM - I,200mg/kg (p.o.)body weight)

Group IV - (STZ-Nicotinamide (i.p)+PHM - II,800 mg/kg (p.o.)body weight)

Group-V- Standard group (STZ-Nicotinamide (i.p) +Pregablin (50) mg/kg body weight (p.o.) Metformin, (200)mg/kg (p.o.) body weight)

The test drug formulations were administered orally using oral gavage tube once daily for 21 days, body weight was checked throughout the experiment, the animals were subjected to eddy's hot plate and tail flick method and sciatic nerve ligation prior one week to the last day of administration **[18]**.

- **Animal method used to determine the analgesic effect**

- **Eddy's hot plate method**

The albino wistar rats used in this investigation, which were male and weighed 150-200 grammes, were experimentally inexperienced. The hot plates were maintained at a temperature of 55 to 56 degrees Celsius. The rat was first placed in the cabin and observe the behaviours include jumping, paw withdrawal, and paw licking were noted. The time between placing the mice and when they responded was timed using a timer (latency period) for 9 hours with the time interval of 1 hour and they were noted **[19]**.

- **Cold allodynia method**

During this behavioural experiment, the rat's right hind paw was immersed in ice-cold water that was kept at a temperature of 4 1°C. We looked at the rat's paw withdrawal delay. 20s was the maximum cutoff time **[20]**.

- **Tail immersion method**

The tail immersion strategy was used to examine the main mechanism of the analgesic effect. The animals' tail tips were placed in hot water to create thermal stimulation. To gauge the baseline reaction time, the rats tail ends (the last 1-2 cm) were immersed in hot water heated to (55± 1) °C at regular intervals of 1 hour for continuous 9 hours. The withdrawal of tail was noted **[21]**.

Sciatic nerve ligation method

Induction of Peripheral Neuropathy by Partial Sciatic Nerve Ligation (PSNL)

The albino wistar rats were put to sleep using thiopental sodium anaesthesia (35 mg/kg, i.p.). The rat's lower back between its thighs and right hind leg were both shaved. 70% isopropyl alcohol was used to sanitise the region after shaving. The skin was removed from the muscle around the incision, which was made 3–4 mm below the femur on the right thigh. The rat's sciatic nerve was exposed by making a straight cut through its muscles. With chromic gut 4.0, a single ligation of roughly 1/3 to 1/2 of the sciatic nerve's diameter was carried out. Mersilk 5.0 was used to secure the skin, and catgut 4.0 or chromic gut 4.0 sutures were used to close the open muscle layer. Povidone-iodine solution was then applied to sterilise the wound and antibiotic powder (Clocip) was applied on the wound as well.

Percentage protection against thermal stimulus

$$\text{Percentage protection against thermal stimulus} = \frac{\text{Test mean (Ta)} - \text{Control mean (Tb)}}{\text{Control mean (Tb)}} \times 100.$$

Figure 1: Sciatic nerve

Figure 2: After sciatic nerve ligation

RESULTS

ANTIHYPERGLYCEMIC RESULTS

THE EFFECT OF VARIOUS GROUPS OF FASTING BLOOD SUGAR

Table 1, represents the fasting blood sugar of 0th day. In the present study the fasting blood sugar of all the animals are considered between 280- 340mg/dl. The mean fasting blood sugar of different groups such as NC, DC, Standard, PHM 1, PHM 2, PHM3 is found to be 86.33±1.9,216.5± 0.8, 214.33±1.3, 218.33±0.7, 218±0.5, 216.66±1.4 respectively.

Table 2, represents the fasting blood sugar on 21stday. Where as the fasting blood sugar of disease control group is found to be increased i.e.,362.5±3.3. the fasting blood sugar of standard, PHM1, PHM2, PHM3after a group of successive treatment for 21 days is found to be 106.33±3.5, *167.66±4.4., *167.66±4.4, **151.73±4.5, 122.5±3.0 respectively. The statistical analysis is done by Bonferroni post hoc test two-way ANNOVA, level of Significance, p value is found to be *p<0.05 when comparing PHM 1 with disease control, p value is found to be**p<0.01, when

comparing PHM2 with disease control p value is found to be p<***0.001when comparing PHM3 with disease control.

RESULTS OF DIABETIC NEUROPATHY ACTIVITY (Table 3, 4)

Table 5, Statistical analysis: Data was analysed by Graph pad prism software version 5.01. Comparision between different groups was done by ANOVA followed by Bonferroni post test.

Figure 5, Statistical analysis: Data was analysed by Graph pad prism software version 5.01. Comparision between different groups was done by ANOVA followed by Bonferroni post test.

Pain latencies were comparable in all the groups. Analgesic activity in PHM1 is statistically significant when compared to control group, (p<0.001***). Analgesic activity in PHM1 is statistically significant when compared to disease controlgroup, (p<0.01**).

Figure 6,S tatistical analysis: Data was analysed by Graph pad prism software version 5.01.Comparision between different groups was done by ANOVA followed by Bonferroni post test.

Pain latencies were comparable in all the groups. Analgesic activity in PHM1 is statistically significant when compared to control group, (p<0.05*). Analgesic activity in PHM1 is statistically significant when compared to standard group, (p<0.001**).

Figure 8, Effect of different doses on the treatment of PHM and Pancreas of histology analysis in Nicotinamide Streptozotocin induced Diabetic rats. A: Normal, B: Standard (Metformin) F: Phm 3 C: Phm-1, D: Phm-2, E: Control.

Table 1: The FBS levels on 7th day

GROUP	R1	R2	R3	R4	R5	R6	MEAN ±SEM
NC	85	80	85	82	98	88	86.3±2.5
DC	217	220	215	216	214	217	216.±0.8
STANDARD	215	220	215	214	212	210	#214.33±13
PHM1 (H.D)	218	219	220	218	215	220	218.33±0.7
PHM2 (M.D)	216	218	219	217	220	218	218±0.5± 0.5
PHM3 (L.D)	210	218	218	219	216	219	**216.66±1.4

Table 2: The FBS levels on 21st day on induction

GROUP	R1	R2	R3	R4	R5	R6	MEAN±SEM
NC	73	82	79	87	78	81	80±1.8
DC	360	359	355	370	375	356	#362.5±3.3
STANDARD	95	100	105	108	110	120	106.33±3.5
PHM1(H.D)	167	166	167	169	168	169	*167.66±4.4
PHM2(M.D)	150	152	151	151.2	153	15 3.2	**151.73±4.5
PHM3 (L.D)	110	120	130	120	125	130	***122.5±3.0

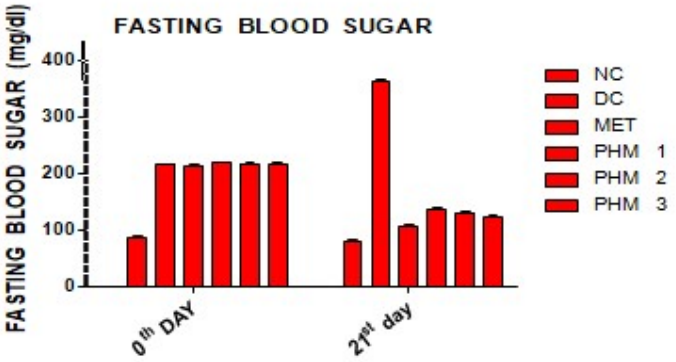

Figure 3: Estimation of blood sugar after induction

Table 3: Eddy's hot plate method on 0th day (n=6)

GROUP	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs
NC	2.49	2.50	2.49	2.51	2.48	2.13	2.12	2.15	2.16
DC	4.23±0.14	4.67±0.24	3.87±0.01	3.76±0.08	5.12±0.09	4.32±0.10	4.11±0.23	3.14±0.06	4.67±0.04
METFORMIN + PREGABLIN	4.56± 0.08	5 .67±0.1	4.89 ±0.2	6.34±0.03	8.54±0.04	10.11±0.02	13.05±0.06	15.44±0 .04	#17.37±0. 1
PHM1	**3.54±0. 03	**4.64±1.89	**5.34±1. 54	**7.89± 1.08	**7.45±2. 09	**8.43±1. 06	**9.68±2. 09	**10.54 ±0.01	**13.68± 2.2
PHM2	2.89±0.86	3.87±0.06	4.15±0.24	4.38±0.26	5.37±0.89	6.78±0.10	7.89±0.69	8.38±0. 84	9.42±0.96

Table 4: Eddy's hot plate method on 21st day

GROUP	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs
NC	2.49	2.50	2.49	2.51	2.48	2.13	2.12	2.15	2.16
DC	3.13 ± 0.04	4 .56±0.25	3.45±0.0 7	3.67±0.0 7	4.34±0.0 6	4.68±0.0 8	4.58±0.1 0	4.25±0.0 7	3.68±0.0 5
METFORMIN + PREGABLIN	#18.57± 0.09	#20.38± 0.05	#23.87± 0.03	#25.37± 0.05	#26.26± 0.04	#26.68± 0.06	#25.78± 0.07	#23.15± 0.08	#22.08± 0.09
PHM1	**15.89 ±0.06	**16.47 ±0.26	**17.48 ±0.37	**18.59 ±0.07	**18.89 ±1.09	**16.47 ±0.04	**15.58 ±0.47	**15.89 ±0.07	**14.68 ±0.23
PHM2	12.67±0. 89	14.67±0. 47	14.98±0. 59	15.67±0. 76	16.38±0. 26	17.67±0. 04	18.67±0. 48	19.58±0. 69	20.42±0. 59

Table 5: Percentage protection on 21st day by Eddy's hot plate

Groups	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	9 hr
METFORMIN + PREGABLIN	#86.59± 0.54	#87.73± 0.54	#89.56± 0.56	#90.10± 0.56	#90.55± 0.78	#92.01± 0.89	#91.77±0.45	#91.44± 0.87	#90.21± 0.23
PHM-1	**84.41 ±0.67	**84.88 ±0.78	**85.75 ±0.67	**86.60 ±0.45	**86.28 ±0.34	**80.99 ±0.67	**81.33±0.5 4±0.75	**80.42 ±0.56	**79.23 ±0.56
PHM-2	80.34±0. 56	82.95±0. 54	83.33±0. 76	83.98±0. 32	84.85±0. 67	82.33±0. 87	79.91±0.54	76.11±0. 21	74.11±0. 76
DC	20.44±0. 67	45.17±0. 43	27.82±0. 87	31.60±0. 54	43.08±0. 21	54.48±0. 54	53.71±0.31	49.41±0. 65	41.30±0. 54

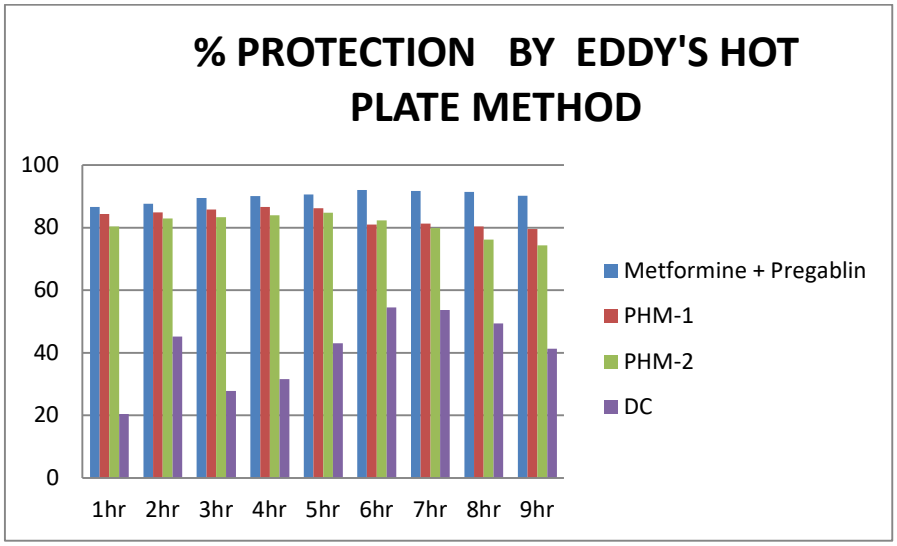

Figure 4: Percentage Protection By Eddy's Hot Plate Method

Pain latencies were comparable in all the groups. Analgesic activity in PHM1 is statistically significant when compared to control group, (p<0.01). Analgesic activity in PHM1 is statistically significant when compared to standard group, (p<0.05*)**

Table 6: Hot water immersion method on 0th day (n=6)

GROUP	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs
NC	2.76	2.45	2.89	3.12	2.89	2.24	2.78	3.45	3.28
DC	3.89±0.38	3.37±0.74	5.67±0.29	4.58±0.97	5.78±0.39	4.78±0.56	4.46±0.57	3.86±0.04	2.78±0.43
METFORMIN + PREGABLIN	#10.13±o.48	#12.78±0.05	#11.57±0.01	#15.68±0.05	#17.49±0.08	#19.25±0.02	#21.05±0.03	#22.38±0.06	#22.67±0.02
PHM1	**8.68±0.48	**9.56±0.19	**10.42±1.62	**10.89±0.59	**11.58±0.54	**12.04±0.04	**14.78±0.34	**15.78±0.07	**16.06±0.42
PHM2	7.89±0.43	8.94±0.67	9.32±0.76	9.67±0.01	10.79±0.07	11.37±0.02	12.67±0.03	12.95±0.67	13.65±0.05

Table 7: Hot water immersion method on 21st day

GROUP	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs
NC	2.76	2.45	2.89	3.12	2.89	2.24	2.78	3.45	3.28
DC	3.67±0.02	4.58±0.56	3.16±0.86	4.46±0.19	4.16±0.75	4.67±0.45	5.37±0.76	4.17±0.78	3.36±0.65
METFORMIN + PREGABLIN	#15.17±0.56	#17.57±0.87	#18.58±0.67	#21.58±0.65	#22.48±0.83	#22.68±0.06	#21.69±0.46	#20.48±0.06	#19.12±0.01
PHM1	**13.78±0.56	**14.78±0.56	**16.58±0.12	**18.37±1.8	**18.59±0.29	**19.45±2.2	**17.98±0.56	**18.94±0.53	**18.02±0.34
PHM2	11.67±0.42	12.53±0.41	13.69±0.32	15.05±0.54	16.78±0.02	17.94±0.34	15.93±0.42	14.32±0.42	13.73±0.27

Table 8: Percentage protection on 21st day by hot water immersion method

Groups	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	9 hr
METFORMIN + PREGABLIN	#81.60±0.76	#86.60±1.5	#84.44±0.67	#85.54±0.87	#87.14±0.87	#90.12±0.67	#87.18±0.76	#83.15±0.34	#82.84±0.43
PHM-1	**79.97±0.78	**83.42±0.89	**81.45±0.19	**82.56±0.72	**83.01±0.23	**88.48±0.87	**84.53±0.76	**81.78±0.76	**80.37±0.56
PHM-2	76.34±0.77	80.44±0.23	74.88±1.5	79.26±0.76	82.77±0.54	87.51±0.43	82.54±0.76	75.90±0.12	74.11±0.65
DC	04.26±0.32	34.32±0.65	36.82±0.84	18.95±0.65	18.72±0.56	12.16±0.87	08.44±0.89	33.91±0.21	42.66±0.76

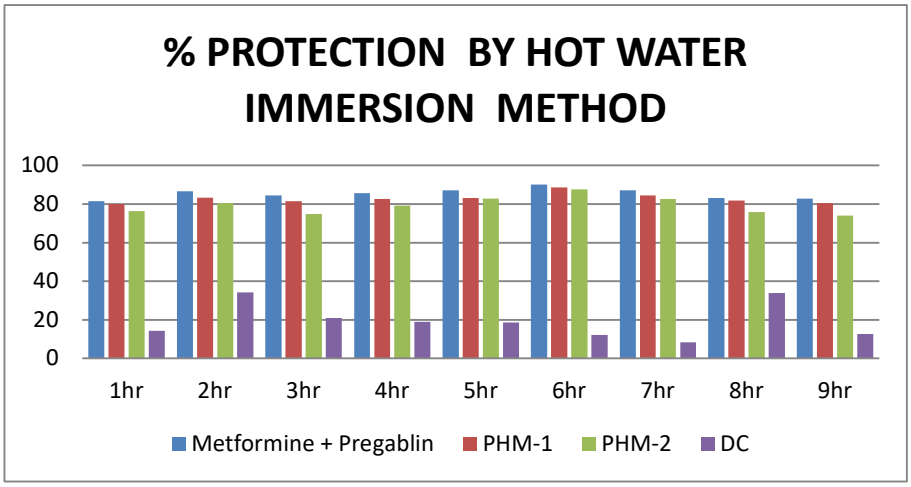

Figure 5: Percentage Protection By Hot Water Immersion Method

Table 9: Cold allodynia method on 0th day

GROUP	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs
NC	3.32	3.67	4.54	3.78	4.38	5.69	4.63	3.27	4.37
DC	4.67±0.53	4.52±0.02	2.76±0.04	2.45±0.23	2.89±0.03	3.12±0.54	2.89±0.34	2.24±0.45	2.78±0.02
METFORMIN + PREGABLIN	#8.78±0.45	#10.32±0.01	#12.98±0.05	#14.01±0.46	#15.39±0.88	#17.26±0.16	#18.38±0.19	#19.42±1..2	#20.05±0.06
PHM1	**7.47±0.64	**8.35±0.87	**9.13±0.78	**10.04±0.54	**11.51±0.12	**12.62±0.56	**13.36±0.03	**14.67±0.34	**16.62±0.34
PHM2	6.56±0.34	7.01±0.72	8.98±0.42	9.52±0.12	10.25±0.76	11.58±0.65	12.73±0.34	12.93±0.37	13.84±0.37

Table 10: Cold allodynia method on 21st day

GROUP	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs
NC	3.67	2.87	3.67	2.78	3.56	3.78	3.36	2.67	2.62
DC	3.52±0.13	4.37±0.24	3.67±0.52	3.43±0.21	4.38±0.24	3.37±0.73	3.67±0.18	4.04±0.34	4.57±0.19
METFORMIN + PREGABLIN	#12.36±0.53	#15.67±	#17.62±0.35	#19.04±1.5	#20.56±1.8	#19.58±0.38	#18.57±0.74	#17.58±0.45	#16.68±0.24
PHM1	**9.45±0.34	**10.28±0.56	**10.67±0.54	**11.89±0.32	**12.93±0.86	**11.59±0.86	**10.69±0.24	**8.78±0.14	**6.89±0.21
PHM2	6.79±0.64	8.05±0.46	9.45±0.86	10.05±0.78	11.67±0.10	10.39±0.86	8.89±0.68	7.69±0.76	5.99±3±0.75

Table 11: Percentage protection on 21st day by cold allodynia method

Groups	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	9 hr
METFORMIN + PREGABLIN	#70.30±0.34	#81.68±o.55	#79.17±0.75	#85.39±1.5	#87.54±0.65	#80.69±0.65	#81.95±0.87	#78.42±0.23	#77.32±0.78
PHM-1	**61.16±0.66	**72.08±0.23	**65.66±0.87	**76.61±0.97	**80.20±0.15	**67.38±0.76	**68.56±0.98	**69.58±1.5	**61.97±0.57
PHM-2	45.94±0.86	64.34±0.45	61.16±0.76	69.23±0.75	78.06±0.14	63.61±0.76	62.20±0.97	61.27±0.86	56.26±0.78
DC	24.79	46.50	18.54	30.04	30.52	52.03	48.23	17.26	12.38

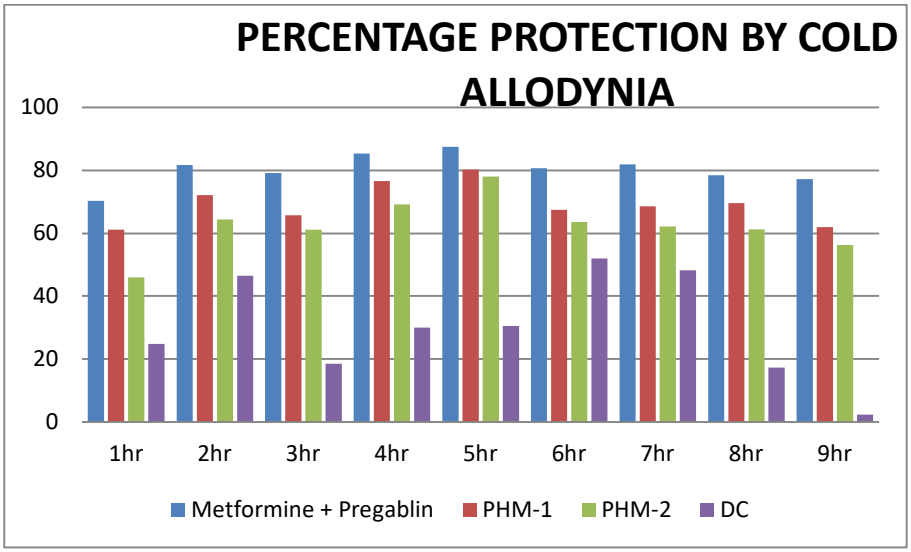

Figure 6: Percentage Protection By Cold Allodynia

Table 12: Hot water immersion method on 0th day (n=6)

GROUP	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs
NC	2.76	2.45	2.89	3.12	2.89	2.24	2.78	3.45	3.28
DC	3.89±0.38	3.37±0.74	5.67±0.29	4.58±0.97	5.78±0.39	4.78±0.56	4.46±0.57	3.86±0.04	2.78±0.43
METFORMIN + PREGABLIN	#10.13±o.48	#12.78±0.05	#11.57±0.01	#15.68±0.05	#17.49±0.08	#19.25±0.02	#21.05±0.03	#22.38±0.06	#22.67±0.02
PHM1	**8.68±0.48	**9.56±0.19	**10.42±1.62	**10.89±0.59	**11.58±0.54	**12.04±0.04	**14.78±0.34	**15.78±0.07	**16.06±0.42
PHM2	7.89±0.43	8.94±0.67	9.32±0.76	9.67±0.01	10.79±0.07	11.37±0.02	12.67±0.03	12.95±0.67	13.65±0.05

Table 13: Hot water immersion method on 10th day

GROUP	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs
NC	2.76	2.45	2.89	3.12	2.89	2.24	2.78	3.45	3.28
DC	3.67±0.02	4.58±0.56	3.16±0.86	4.46±0.19	4.16±0.75	4.67±0.45	5.37±0.76	4.17±0.78	3.36±0.65
METFORMIN + PREGABLIN	#15.17±0.56	#17.57±0.87	#18.58±0.67	#21.58±0.65	#22.48±0.83	#22.68±0.06	#21.69±0.46	#20.48±0.06	#19.12±0.01
PHM1	**13.78±0.56	**14.78±0.56	**16.58±0.12	**18.37±1.8	**18.59±0.29	**19.45±2.2	**17.98±0.56	**18.94±0.53	**18.02±0.34
PHM2	11.67±0.42	12.53±0.41	13.69±0.32	15.05±0.54	16.78±0.02	17.94±0.34	15.93±0.42	14.32±0.42	13.73±0.27

Table No.12: Percentage protection on 10th day by sciatic nerve ligation method

Groups	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	9 hr
METFORMIN + PREGABLIN	#81.60±0.76	#86.60±1.5	#84.44±0.67	#85.54±0.87	#87.14±0.87	#90.12±0.67	#87.18±0.76	#83.15±0.34	#82.84±0.43
PHM-1	**79.97±0.78	**83.42±0.89	**81.45±0.19	**82.56±0.72	**83.01±0.23	**88.48±0.87	**84.53±0.76	**81.78±0.76	**80.37±0.56
PHM-2	76.34±0.77	80.44±0.23	74.88±1.5	79.26±0.76	82.77±0.54	87.51±0.43	82.54±0.76	75.90±0.12	74.11±0.65
DC	04.26±0.32	34.32±0.65	36.82±0.84	18.95±0.65	18.72±0.56	12.16±0.87	08.44±0.89	33.91±0.21	42.66±0.76

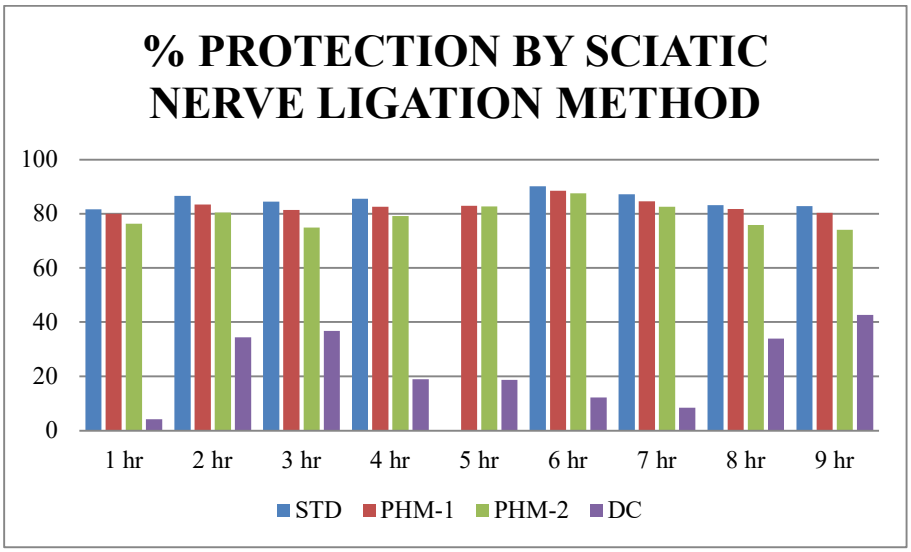

Figure 7: Percentage Protection By Cold Allodynia

HISTOPATHOLOGY STUDIES

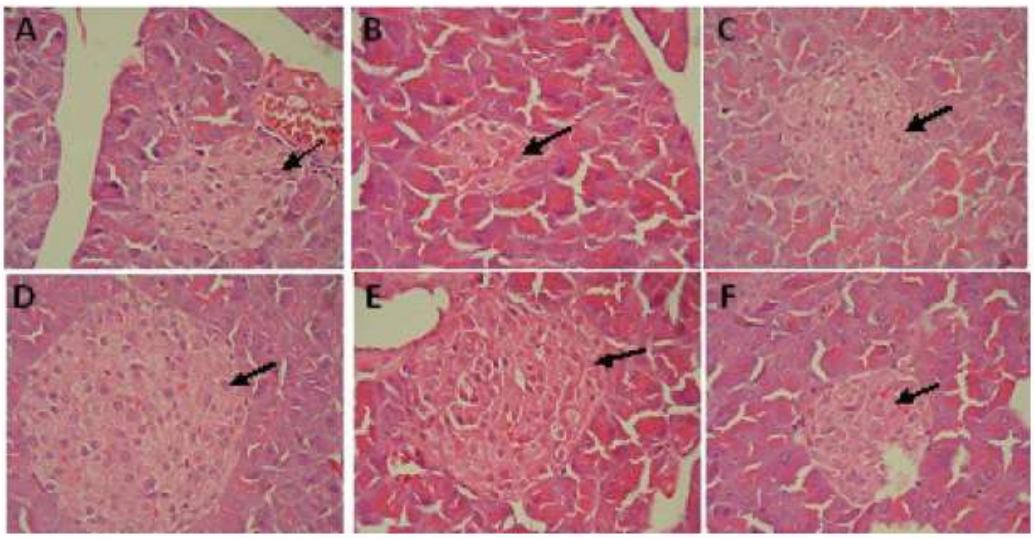

Figure 8: Histopatology Study of Pancreas

DISCUSSION:

This favourable impact on glycemic status is anticipated, given the antihyperglycemic effects of all 3 plants that make up PHM have been repeatedly demonstrated in research. Inhibiting glucose production in the liver, increasing pancreatic tissue regeneration, improving insulin secretion from beta cells, increasing glucose uptake by tissues, and the presence of insulin-like agents in plants are just a few of the mechanisms by which medicinal plants produce their antihyperglycemic effects **[22]**.

An important risk factor for cardiovascular disorders, dyslipidemia, is frequently linked

to diabetes. So, in diabetic patients, blood triglyceride and cholesterol levels are typically higher. In our investigation, PHM was able to reduce hypercholesterolemia and serum triglyceride levels. PHM's hypolipidemic effects are consistent with other research that found *Ocimum tenuiflorum*, *Syzygium cumini* and Aegel *Marmelos* reduce serum triglyceride and cholesterol levels in diabetic patients **[23]**.

Together, the data showed that PHM has positive impacts on diabetic rats' blood glucose and lipid profiles. Using a polyherbal combination of *Ocimum tenuiflorum*, *Syzygium cumini* and Aegel *marmelos* macerated and Soxhlet (hydroalcoholic) extracts, as a result, we discover beneficial effects on diabetes when combining 3 plants to create hydroalcoholic extracts of 3 of these plants. Additionally, several research found that giving specific doses of herbal extracts or formulations, particularly *Ocimum tenuiflorum*, had a greater antihyperglycemic impact **[24]**.

Combining different hypoglycemic and hypolipidemic herbs to create a more potent antidiabetic drug is one method for creating an effective phytochemical combination. However, when administered, the antidiabetic effect was typically attained with low doses of the plant extract, which may be accompanied by undesirable side effects in the body. In light of this, had the potential to be utilised as a dietary supplement for the management of diabetes, the significant p value of diabetic activity when compared to disease control was **p< 0.01 its order was seen as PHM-3 > PHM-2 > PHM-1 .The PHM was also used for analgesic activity which shown great response on the rats which were clearly examined by using the hot water immersion , eddy's hot plate method and cold allodynia method and sciatic nerve ligation method which has the activity to withhold the pain by administrating to the rats. The main constituent which was responsible for analgesic activity in the PHM was carvacrol. The significant p value of analgesic activity when compared to disease control**p<0.01 with PHM1 and was seen as PHM-1> PHM-2. Clearly, additional research is required to identify a more potent polyherbal blend from anti-diabetic herbs and also on analgesic activity in a future view point connected to the management of diabetes and complication.

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