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HYPOGLYCEMIC ACTIVITY PROFILE OF POLYHERBAL FORMULATION (PF) AND POTENTIATION OF HYPOGLYCEMIC EFFECT OF METFORMIN IN RODENTS

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Polyherbal formulation, Metformin, Antidiabetic activity, Glucose metabolism, Glucose utilization

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ABSTRACT: Polyherbal formulation (HF) containing different herbs have been used to treat diabetic patients by ayurvedic practitioners in India. The present study reports the effect of HF alone and with metformin on different rat models of hyperglycemia. HF treatment with HF alone and in combination with metformin elicited a significant hypoglycemic effect on epinephrine hyperglycemia and alloxan diabetic rats. HF treatment also altered the glucose-tolerance curve pattern both in normal and diabetic rats. Further, HF treatment alone and with metformin increased liver glycogen, glucose transfer in the liver, enhanced glucose uptake process in peripheral muscles, and also improved architecture of β-islets of Langerhans of diabetic rats. The combination of HF with metformin elicited a greater degree of hypoglycemic activity, which was reflected both on blood glucose level as well as other biochemical parameters studied. The hypoglycemic effect of HF may be mediated through pancreatic as well as extrapancreatic systems. Further, HF combination with metformin seems to be useful in diabetes mellitus and needs to be investigated on human subjects to establish such potentiation hypoglycemic effect of metformin.

INTRODUCTION: Diabetes mellitus (DM) is a group of metabolic disorders characterized by abnormalities in carbohydrate and lipid metabolism [1], which leads to hyperglycemia, hypertri-glyceridemia and hypercholesterolemia resulting from defects in insulin secretion or action or both [2]. The disease is managed clinically with insulin and oral hypoglycemic agents like sulphonylureas and biguanides. The biguanide phenformin was withdrawn from US market in 1997 following reports of the relatively high incidence of lactoacidosis [3] and later replaced by metformin.

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These drugs are too expensive, and thus, beyond the reach of diabetes mellitus (DM) patients, especially in developing countries. Oral hypoglycemic drugs play an important role in the treatment of non-insulin-dependent DM. But none have been unequivocally successful in maintaining euglycemia and avoiding complications during diabetes. One of the late complications of uncontrolled DM is the excessive formation of advanced glycosylated end products (AGE) [4].

Despite several advances in therapeutics and a better understanding of the disease, diabetes remains a significant cause of morbidity and mortality [5]. Ancient Indian medicine recommends various plants and mineral preparation in the treatment of DM. Herbal medicines have stood the test of time for their safety, efficacy, cultural acceptability, and lesser side effects.

The constituents may precipitate interaction *via* metabolism and are also known to alter the duration as well as onset of pharmacological effects of components in multiple drug therapy [6]. Herbo-mineral formulation (HF) containing *Syzgium cumini, Annona squamosa, Momordica charantia, Tinospora cordifolia, Gymnema sylvestre,* and *Curcuma longa* as its main ingredients (see **Table 1**) and are found to be safe [7]. Hypoglycemic-antidiabetic activity evaluation in experimental hypoglycemic models and whether HF potentiates the hypoglycemic effect of metformin along with other beneficial effects on lipids.

TABLE 1: COMPOSITION OF HERBOMINERAL FORMULATION

Herbs	Quality (mg)
Aq. extract of *Tinospora cordifolia*	175
Aq. extract of *Gymnema sylvestre*	150
Aq. extract of *Syzgium cumini*	100
Aq. alcoholic extract of *Annona retculata*	175
Aq. alcoholic extract of *Momordica charantia*	150
Alcoholic extract of *Curcuma longa*	100

MATERIALS AND METHODS:

Drugs, Phytochemicals and Other Materials: A herbal extract procures from Green Phyto Chem Pvt. Ltd. Bangalore, Metformin (Cipla, India), was obtained as gift samples. Epinephrine tartrate injection (Xion Tamil Nadu, India), Diagnostic Kits (Biolab, Mumbai) were purchased.

Animals: Wistar rats of either sex, weighing 200–250 g were purchased from Intox Pvt. Ltd.Pune, India Rats were housed in our animal house under standard conditions: temperature 24° ± 1 °C, RH 65±10%, light-dark cycle (10:14h), and fed with standard pellet diet water *ad libitum*. All animals were handled with humane care.

Ethics Committee Approval: The experiment protocol was approved by the Institutional Animal Ethical Committee of Dr. D. Y. Patil College of Pharmacy, Akurdi Pune (DYPCOP/IAEC/2019-01-01) in accordance with the guidelines of IPCSEA.

Preparation of Test and Reference Drug: Suspension of finely powdered HF and metformin were prepared in 1% (w/v) sodium carboxymethyl cellulose (CMC) and administered orally. Alloxan was prepared in ice-cold normal saline freshly prior to administration.

Hypoglycemic Activity Screening in Rats:

Effect on Normal Fasted Rats: Normal Wistar rats were used for evaluation of HF for hypoglycemic activity. Rats fasted overnight were randomly divided into 5 groups (6 rats/ group), and the test and reference drugs were administered orally. Group 1 served as control and received 0.5ml/100g of 1% CMC solution. Group II, III, and IV were treated with HF 225, 450, and 850 mg/kg orally, respectively, and Group V received metformin (500 mg/kg) orally. Blood samples were collected at 0, 2, 4, and 6 h after the respective treatment by puncturing retro-orbital plexus under mild ether anesthesia using fine glass capillary in Eppendorf tubes. Blood glucose concentration was assayed by a glucose oxidase method using contour blood glucose strips [8].

Effect on Glucose Loaded Normal Rats: The oral glucose tolerance test was carried out to find out responses appropriately to a glucose challenge. Rats fasted overnight were randomly divided into 5 groups (6 rats/group). The rats of different group were administered (0.5ml/100g of 1% CMC) vehicle, HF (225, 450 and 850 mg/kg) and metformin (500 mg/kg) orally to different groups (6 rats/group). Rats of all these groups received glucose (2g/kg) orally. Blood samples were collected puncturing retro-orbital plexes before drug administration at 0.5, 1, and 2 h (after glucose administration) for blood glucose determination [9].

Effect on Epinephrine Induced Hyperglycemic Rats: Overnight fasted rats were randomly divided into 5 groups (6/group) after determining their fasting blood glucose levels. The Groups I served as hyperglycemic control and received vehicle (0.5ml/100g, po). Rats of Group II, III, and IV were treated with HF (225, 450, and 850 mg/kg), orally respectively. Group V and Group VI rats received metformin (500 mg/kg, po). Epinephrine hydrochloride (0.8 mg/kg) was administered intra-peritoneally (ip) to rats of all groups 2 h after the vehicle, HF, and metformin administration. Blood glucose levels were determined at 0.5, 1, and 2 h after epinephrine treatment [10].

Induction of Experimental Diabetes: Experimental diabetes was induced in rats by injecting alloxan monohydrate (150 mg/kg ip). Alloxan dissolved in citrate buffer at pH 4.5 and

injected immediately within few minutes to avoid degradation. After a week, blood was collected from the retro-orbital plexus under mild ether anesthesia. Rats with fasting blood glucose levels showing more than 250 mg/dl were selected for study [11].

Anti-diabetic Activity Screening: Diabetic rats were divided into 5 groups (6/group). Group I and II rats served as normal and diabetic control, respectively, and received a vehicle (0.5ml/100g) orally. Group III and IV received HF (225 mg/kg), metformin (250 mg/kg) orally, respectively. Group V received HF (225 mg/kg) + metformin (250 mg/kg) orally on the 7th day after alloxan treatment.

In Single-Dose Study: To find out the single-dose effect on blood glucose in alloxan diabetic rats was determined before treatment and at 2, 4, 6 h after the administration of test drugs and vehicle.

Sub-chronic Study (21 Days): HF (225mg/kg), metformin (250 mg/kg) and HF (225 mg/kg) + metformin (250mg/kg) were administered orally daily to alloxan diabetic rats for 21 days. Blood glucose level was determined on 3rd, 5th, 7th, 14th and 22nd day. (Blood was collected 24 h after the last dose) during the treatment period [12].

Biochemical Investigation: On the 21nd day, rats were sacrificed, and blood was collected by cardiac puncture under light ether anesthesia for various biochemical estimations. The serum was separated by centrifuging the sample at 3000 rpm for 5 min. The serum was analyzed for total cholesterol (CHOD-PAP method), HDL, triglyceride (GPO method). Glycosylated hemoglobin (GHb %) was determined in whole heparinized blood using the ion-exchange resin method [13] using a commercial kit from vector Biotek Pvt. Ltd. Gujrat India. Subsequently, the Hb A/C fraction and mean blood glucose were calculated from the GHb %. The liver, diaphragm, and pancreas were dissected out and washed with ice-cold saline immediately and preserved at -5 °C in a refrigerator.

Effect of Glucose Uptake by Hemidiaphragm of Diabetic Rats: The hemidiaphragm of rats were placed in ice-cooled perfusion solution (Tyrodesolution) with the following composition: NaCl (0.687%), KCl (0.04%), $MgSO_4$ (0.014%), $CaCl_2$ (0.028), $NaHPO_4$ (0.014%) and $NaHCO_3$ (0.21%). Glucose (400 mg %) solution was prepared in a perfusion solution and added to the perfusion solution. The hemidiaphragm was incubated at 37 °C ± 0.5 °C in Tyrode solution with glucose (400 mg %) for 1 h with appropriate aeration. At the end of the incubation period, glucose concentration in the perfusate was determined [14]. The diaphragm was removed, rinsed in water, and dried in an oven at 60 °C for 4-5 h or till a constant weight was obtained. The glucose uptake was expressed in terms of mg/100 mg dry weight of the diaphragm.

Effect on Liver Glucose Transfer Process in Diabetic Rats: Livers of rats were sliced (by cutting with a razor blade) then incubated at 37 °C ± 0.5 °C in Tyrode solution with 2% glucose for 1 h [14]. The glucose content in the perfusate after 60-minute incubation was determined. The dry weight of the liver was calculated by drying liver slices in an oven at 60 °C until a constant weight of the liver was obtained. The results were expressed as glucose transfer mg/hr/gm of the dry weight of the liver [14, 15].

Effect on Liver Glycogen Content of Diabetic Rats: The liver was homogenized in 5% (w/v) trichloroacetic acid, and its glycogen content was determined by method [15].

Histopathological Studies: The pancreas was isolated carefully and preserved in 10% formalin. Pancreas stored in 10% formalin were embedded in paraffin section cut out at 5 μm and stained with hematoxylin and eosin. These sections were then examined under light microscopy for histo-architectural changes. Histopathological studies were carried out at Padro Laboratories Pvt. Ltd. Pune.

Statistical Analysis: The results are expressed as mean ± SEM. The results of different experiments were subjected to statistical analysis using one-way ANOVA following by Dunnet's multiple comparison test for control groups; the p-value <0.05 was considered to be significant.

RESULTS:

Hypoglycemic Activity Screening in Rats:

Normal Rats: After the administration of HF (225, 450 and 850 mg/kg p.o.) the onset of hypoglycemic effect was observed between 2 to 4 h, and peak was

found to be at 4 h. Administration of herbomineral formulation (450 & 850 mg/kg p.o.) significantly (P<0.05) decreased blood glucose level as compared to the normal control group **Table 2**. Metformin (500 mg/kg p.o.) treated rats did not elicit hypoglycemic effect in normal rats.

TABLE 2: HYPOGLYCEMIC EFFECT OF SINGLE DOSE ADMINISTRATION OF HF ON BLOOD GLUCOSE LEVEL IN NORMAL RATS

Treatment (mg/kg, p.o.)	Blood glucose level (mg/dl) at different time intervals Mean ± SEM (n-6)			
	0 h	2 h	4 h	6 h
Normal Control	58.9 ± 2.8	58.4 ± 2.5	58.8 ± 1.8	59.6 ± 2.6
HF (225)	63.2 ± 2.1	58.9 ± 2.2	47.5 ± 4.3*	56.2 ± 3.9
HF (450)	55.4 ± 5.3	49.7 ± 3.8*	45.8 ± 3.8*	51.7 ± 4.8*
HF (850)	54.6 ± 5.6	47.2 ± 3.6*	41.3 ± 4.4*	50.2 ± 2.1*
Metformin (500)	59.0 ± 2.9	50.5 ± 2.9*	50.5 ± 1.5*	50.2 ± 3.1*

*P<0.05 compared to the normal control group

Effect of HF, Metformin and their Combination on Glucose Loaded Normal Rats: The result of the glucose tolerance test conducted on normal rats fed with various doses of herbomineral formulation is outlined in **Table 3**. The pretreatment with HF (225, 450, & 850 mg/kg) and metformin (500 mg/kg) prevented the increased blood glucose level significantly at all intervals studied.

TABLE 3: EFFECT OF SINGLE DOSE ADMINISTRATION OF HF, ON BLOOD GLUCOSE LEVEL IN GLUCOSE LOADED RATS

Treatment (mg/kg, p.o.)	Blood glucose level (mg/dl) at different time intervals Mean ± SEM (n-6)			
	0 h	0.5 h	1 h	2 h
Normal Control	75.4 ± 2.8	203.8 ± 9.3	147.1 ± 4.7	114.8 ± 6.9
HF (225)	75.7 ± 3.5	128.60 ± 17.0*	108.8 ± 6.9$^{\#}$	98.5 ± 1.7
HF (450)	69.7 ± 12.3	123.80 ± 18.9*	94.37 ± 16.3*	92.25 ± 4.5$^{\#}$
HF (850)	66.5 ± 2.5	104.57 ± 4.5$^{@}$	91.65 ± 4.5*	94.42 ± 5.6$^{\#}$
Metformin (500)	62.5 ± 3.5	108.90 ± 15.6$^{@}$	94.56 ± 10.9*	89.48 ± 7.6$^{\#}$

#P<0.05, *P<0.005, @P<0.0001 compared to the normal control group

Effect of HF on Epinephrine Induced Hyperglycemia: Administration of epinephrine (0.8 mg/kg, ip.) elicited a significant (P<0.001) hyperglycemia in rats. Pretreated with the HF (225, 450, & 850 mg/kg) significantly reduced the increased blood sugar due to epinephrine **Table 4**.

From the above experimental hypoglycemic studies in normal rats, the dose of HF425 mg/kg, found to exhibit moderate hypoglycemic activity. Hence 425 mg/kg was selected for anti-diabetic activity evaluation in alloxan diabetic rats.

TABLE 4: EFFECT OF SINGLE DOSE ADMINISTRATION OF HF ON BLOOD GLUCOSE LEVEL IN EPINEPHRINE INDUCED HYPERGLYCEMIC RATS

Treatment (mg/kg, p.o.)	Blood glucose level (mg/dl) at different time intervals Mean ± SEM (n-6)			
	0 h	0.5 h	1 h	2 h
Normal Control	66.31 ± 2.8	131.5 ± 18.7	217.7 ± 29.2	266.9 ± 7.59
HF (225)	65.34 ± 5.4	81.82 ± 11.2$^{\#}$	147.7 ± 8.3$^{\#}$	169.9 ± 20.4*
HF (450)	72.70 ± 4.4	89.07 ± 3.33$^{\#}$	117.2 ± 16.8*	148.4 ± 13.2*
HF (850)	67.19 ± 5.4	82.76 ± 10.2$^{\#}$	115.2 ± 16.5*	114.0 ± 11.7*
Metformin (500)	68.09 ± 3.9	86.82 ± 5.2$^{\#}$	119.6 ± 7.8*	125.0 ± 4.8*

#P<0.05, *P<0.01 compared to the normal control group

TABLE 5: EFFECT OF CHRONICTREATMENT (21 DAY) WITH HF, METFORMIN AND THEIR COMBINATIONON BLOOD GLUCOSE LEVEL IN ALLOXAN-INDUCED DIABETIC RATS

Treatment (mg/kg, *p.o.*)	Blood glucose level (mg/dl) on different days Mean ± SEM (n=6)				
	3	5	7	14	21
Normal Control	65.3 ± 1.4	68.0 ± 0.9	69.10 ± 2.1	71.3 ± 2.6	69.5 ± 1.5
Diabetic Control	281.0 ± 9.0$^{\#}$	277.8 ± 8.3$^{\#}$	275.6 ± 7.8$^{\#}$	262.2 ± 6.6$^{\#}$	253.3 ± 4.9$^{\#}$
HF (225)	276.8 ± 4.5	264.8 ± 3.3*	247.2 ± 2.3**	212.1 ± 5.4$^{@}$	187.6 ± 6.0$^{@}$
Metformin (250)	270.0 ± 4.7	250.9 ± 5.3*	244.8 ± 2.8**	198.1 ± 5.3$^{@}$	169.6 ± 3.5$^{@}$
HF (225) + Metformin (250)	272.6 ± 5.2	250.5 ± 7.7**	220.7 ± 7.2$^{@}$	172.7 ± 4.3$^{@}$	145.2 ± 3.5$^{@}$

*P<0.05, **P<0.01,$^{@}$P<0.001 compared to the diabetic control group, $^{\#}$P<0.001 compared to the normal control group

Anti-diabetic Activity Screening in Alloxan-Induced Diabetic Rats:

Effect of Single-Dose Treatment: Administration of alloxan (150 mg/kg, *ip*) resulted in a marked increase in blood glucose level (P<0.001). Single-dose treatment of HF (225 mg/kg), metformin (250mg/kg) and their combination (HF 225 mg/kg + Metformin 250 mg/kg) orally decreased blood glucose level at 4 h and 6 h in rats **Table 5**.

TABLE 6: EFFECT OF CHRONIC TREATMENT (21 DAYS) WITH HF, METFORMIN AND IN COMBINATION ON GLYCOSYLATED Hb, CHOLESTEROL AND TRIGLYCERIDE LEVELS IN ALLOXAN-INDUCED DIABETIC RATS

Treatment (mg/kg, *p.o.*)	GHb A1 (mg/g Hb)	GHB Alc (mg/g Hb)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)
	Mean ± SEM (n=6)			
Normal control	0.7 ± 0.2	0.4 ± 0.0	68.00 ± 2.9	59.82 ± 1.71
Diabetic control	1.3 ± 0.4$^{\#}$	0.8 ± 0.0$^{\#}$	112.2 ± 4.1$^{\#}$	121.2 ± 3.3$^{\#}$
HF (225)	1.0 ± 0.2*	0.7 ± 0.0*	94.00 ± 2.7*	103.2 ± 2.2*
Metformin (250)	0.6 ± 0.2*$^{\Delta}$	0.7 ± 0.0*$^{\Delta}$	89.8 ± 4.0*	96.1 ± 1.1*
HF (225) + Metformin (250)	0.6 ± 0.2**	0.6 ± 0.0**	74.2 ± 4.1*	79.5 ± 2.6*

*P<0.05, *ΔP<0.01, **P<0.001, compared to the diabetic control group, #P<0.001 compared to the normal control group

Effect on Biochemical Parameters:

Effect on Glycosylated Hemoglobin, Serum Cholesterol, and Triglycerides: Twenty-one days of treatment with HF (225 mg/kg), Metformin (250 mg/kg) and their combination to alloxan diabetic rats reduced the elevated glycosylated hemoglobin, total serum cholesterol, and triglycerides levels significantly **Table 7**. In diabetic rats, the glucose uptake by diaphragm was decreased as compared to the diaphragm of a normal rat. Hemidiaphragm of rats treated for 21 days with HF (225 mg/kg), Metformin (250 mg/kg), and their combination increased uptake of glucose as compared to untreated diabetic rats **Table 7**. The rats treated with HF (225 mg/kg), metformin (250 mg/kg), and their combination increased liver glycogen content as compared to alloxan diabetic control groups **Table 7**. Alloxan diabetic rats treated with HF (225 mg/kg), metformin (250 mg/kg), and their combination showed enhanced glucose transfer from the liver as compared to untreated diabetic rats **Table 7**. Rats treated with HF + metformin showed a greater degree of glucose transfer from the liver as compared to HF and metformin alone treated groups.

TABLE 7: EFFECT OF CHRONIC TREATMENT (21 DAYS) OF WITH HF, METFORMIN AND THEIR COMBINATION ON LIVER GLYCOGEN GLUCOSE TRANSPORT AND GLUCOSE UTILIZATION IN ALLOXAN-INDUCED DIABETIC RATS

Treatment (mg/kg, *p.o.*)	Glucose uptake by hemidiaphragm (mg/100mg) tissue	Glucose transfer (mg/hr/gm) tissue	Liver glycogen level (g/100g) tissue
	Mean ± SEM (n=6)		
Normal control	10.8 ± 0.6	26.4 ± 1.4	2.3 ± 0.10
Diabetic control	03.2 ± 0.7$^{\#}$	42.0 ± 2.0$^{\#}$	0.64 ± 0.0$^{\#}$
HF (225)	05.7 ± 0.4**	32.1 ± 0.8**	1.2 ± 0.0**
Metformin (250)	06.3 ± 0.3*	28.0 ± 0.9*	1.3 ± 0.0*
HF (225) + Metformin (250)	08.0 ± 0.5*	24.2 ± 2.4*	1.9 ± 0.0*

n=6, values are expressed as mean ± SEM, *P<0.001, **P<0.01 compared to the diabetic control group, $^{\#}$P<0.0001 compared to the normal control group

Histopathological Studies: The normal rats showed no architectural changes in the histology of the pancreas and normal cellular population in the pancreatic islets of Langerhans **Fig. 1A**. Alloxan diabetic rats showed diffused necrotic changes, decreased cellular population of islets of Langerhans, accompanied by a reduction in the size of islets **Fig. 1B**. The group of rats treated with HF (225 mg/kg, p.o.), Metformin (250 mg/kg p.o.) showed diffused necrotic changes of mild to a moderate degree and also a mild reduction in the size and number of the islets of Langerhan **Fig. 1C** & **D**. Rats treated with HF 225 mg/kg and metformin 250 mg/kg restored the numbers of islets with hyperplasia significantly. However, the degree of necrotic changes remained mild to moderate in nature only **Fig. 1E**.

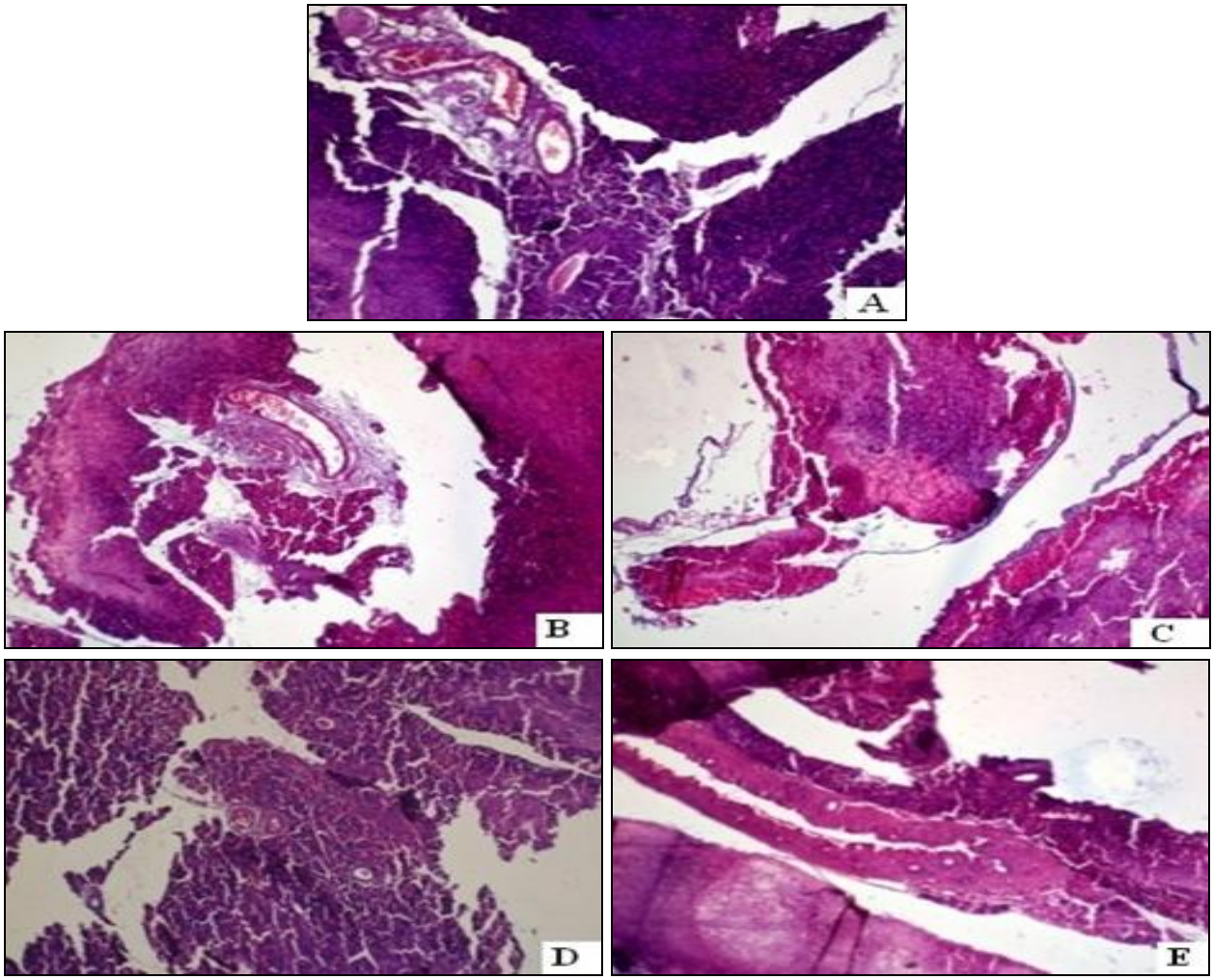

FIG. 1: EFFECT OF HF ALONE, METFORMIN ALONE AND ITS COMBINATION ON HISTOLOGY OF PANCREAS IN ALLOXAN- INDUCED DIABETIC RATS,A) NORMAL CONTROL, B) DIABETIC CONTROL, C) HF (225), D) METFORMIN (250), HF (225) WITH METFORMIN (250) AT 400 X

DISCUSSION: The HF has been used to treat diabetes mellitus by Ayurvedic practitioners in India [16]. Therefore in the present study, HF was evaluated for its antidiabetic activity in rats and, also in combination with metformin to find out whether it potentiate the antidiabetic effect of metformin.

HF per treatment elicited hypoglycemic activity both in normal as well as alloxan diabetic rats. Furthermore, combined treatment with HF and metformin enhanced the anti-hyperglycemic activity of metformin. The hypoglycemic activity o HF was also observed in glucose loaded normal as well as epinephrine induced hyperglycemic rats. Our findings indicate that metformin reduced the blood glucose levels of glucose loaded and epinephrine treated rats but not of normal rats, which is in complete agreement with the earlier reports [14].

The altered blood glucose pattern in glucose-loaded rats was similar to that of sulfonylureas [17]. The observed hypoglycemic effect of HF may be explained in the following way (a) restoration of delayed insulin response or (b) decreased intestinal glucose absorption. Alternatively, the hypo-glycemic effect of HF may be attributed to increased secretion of insulin from intact β-islets of Langerhans or its release from bound insulin [18].

The change in blood glucose concentration could result largely from oral carbohydrate load and is related directly to the rate of absorption in the small intestine, the rate of digestion, and dynamics of the intestine [19]. The predominant effect reported with epinephrine induced hyperglycemia is mainly mediated through the release of glucocorticoids, which affects glucose metabolism in the liver and is also known to inhibit insulin release by the pancreas [20].

Besides, the increased blood glucose levels due to epinephrine has been correlated to decreased uptake of glucose by peripheral tissues, mainly the skeletal muscle [21]. Further, epinephrine is known to enhance glucagon secretion by α-cells of the pancreas and activation of β-receptors resulting in increased glycogenolysis and gluconeogenesis [21]. Therefore the hypoglycemic effect of HF or HF + Metformin may be due to either due to prevention of glucagon secretion from α-cell of the pancreas and/or interference in the activation of β-receptors.

Alloxan selectively destroys pancreatic insulin-secreting β-cells resulting in increased blood glucose levels and finally manifesting into type 2 diabetes mellitus [22]. In the present study, the dose of alloxan (150 mg/kg,ip) was selected because this dose was found to destroy the pancreatic β-cells partially. In such a situation, insulin is secreted from infect β-cells of Langerhan but not adequate enough to regulate the blood glucose levels [23]. In order to understand the mechanism of action responsible for the hypoglycemic activity of HF, we have determined major biochemical events/ pathways in carbohydrate and lipid metabolism during chronic treatment (21 days) of HF, metformin, and HF + metformin to alloxan diabetic rats. Our finding demonstrates a significant reduction in blood glucose levels and increased glycosylated hemoglobin content, and decreased serum cholesterol and triglyceride levels on different days in HF, metformin, and also HF + metformin-treated diabetic rats. The mechanism of action of biguanide is not fully understood. However, few documented reports indicate that metformin enhanced the sensitivity of insulin receptors to augment the glucose utilization process [24].

Glycosylated hemoglobin levels were increased in diabetic rats. Chronic treatment (21 days) with HF, metformin, and HF + metformin to diabetic rats able to decrease the increased glycosylated hemoglobin levels significantly. The inhibitory effect was greater in HF + metformin-treated rats. One of the complications of uncontrolled DM at the later stage is the formation of advanced glycosylated end products [25]. Some of these end products (glycosylated peptides) are known to cause increased permeability and thickening of blood vessel walls with loss of elasticity [25].

Since the average life span of a red blood cell is 120 days, the assumption of clinical use of glycosylated HbA level is that they represent the time-averaged value of blood glucose over 2 to 4 months time period. Hence it provides a useful index in assessing the efficacy of drugs and also patient compliance.

Alloxan diabetes rats exhibited a decreased hepatic glycogen level, which is correlated to the decreased availability of the active form of enzyme, glycogen synthase, largely due to a low level of insulin [26]. Our findings clearly suggest treatment with HF, Metformin, and their combination significantly restored the depleted hepatic glycogen levels. This can be presumed to the enhanced insulin secretion, which in turn able to activate glycogen synthase. In diabetic rats, the glucose uptake process is decreased as compared to normal rats. Treatment of diabetic rats with HF, metformin, and HF + metformin able to restore the decreased glucose uptake process in the peripheral tissues (hemidiaphragm), combined treatment (HF + metformin) elicited a greater effect on glucose uptake process. The action of insulin on its receptors on the plasma membrane is directly linked to glucose transporter proteins, which is depleted during DM. This might explain the elevated glucose level and the decreased glucose uptake by diaphragms of diabetic rats [27].

It has been well documented that in uncontrolled type 2 DM, there is an increased serum total cholesterol, low-density lipoprotein, and triglyceride levels accompanied by decreased high-density lipoproteins [28]. Low-density lipoprotein is known to trigger coronary artery disease [29]. Increased triglycerides and lipolysis in adipocytes are caused by hypo-insulinemia. It is presumed that the repressed plasma insulin levels *via* improvement of insulin resistance with HF treatment, triglycerides store might have reduced and such biochemical events finally result in depletion of lipids [30, 31].

Histopathological findings of the pancreas of the diabetic rats showed necrosis, atrophy, and fibrotic changes. But, the pancreas of the rats treated with HF alone, metformin, or in combination with metformin showed improvement in necrosis (mild to moderate atrophy) and fibrotic changes. Thus,

Histopathological observations are in agreement with biochemical findings in alloxan diabetic rats.

In conclusion, our findings demonstrate that HF exhibits a significant anti-diabetic activity in rats and also enhanced the hypoglycemic activity of metformin. Further, clinical studies on human subjects are required to confirm its ability to potentiate the hypoglycemic activity of metformin.

CONCLUSION: The polyherbal formulation significantly reduces the blood glucose level in normal, hyperglycemic rats. The extract of multi herbs shown to increased insulin secretion from the regenerated pancreatic beta cells. Further study needs to be carried out for clinical assessment and study the pharmacokinetic interactions with modern drugs.

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