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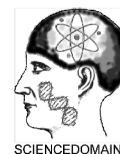

Intrinsic Efficacy of Novel Poly-herbal Formulation Activity on Hypoglycemic, Immune Potentiating and Antioxidant Activities in Diabetic Individual

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SG, RG and NMK designed the study. Author NMK performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author RG managed the analyses of the study. Author RG managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The study was conducted to assess the synergistic poly-herbal formulation for diabetic patients to determine its three characteristics reducing excessive sugar level to normal, immune-potentiating and antioxidant.
Study Design: Subjects were divided into four groups, Group I(NH), were normal healthy subjects, Group II(DI), were diabetics, group III (TTD) were tolbutamide treated diabetic patients, group IV(HFTD), were diabetic patients receiving combination herbal formulation in the, dosage of 5 g. /day for 4 weeks.
Place of Study: Diabetic patients were contacted and convinced from two Government Hospitals-Hamidia Hospital, Bhopal and K. N. Katju hospital, Bhopal, (M.P.), India.
Methodology: homogenous mixtures were obtained and encapsulated 500mg/per capsule. PMNL were isolated from blood and glucose level tests performed by auto-analyzer. Immune- potentiating activity was evaluated by different following methods like

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a ATPase sensitivity tests, cellular water content, cell, plasma membrane calcium content, Camp activity, Phospholipase-C activity, contact angle measurement, NBT assay. Antioxidant activity evaluates by SOD and glutathione peroxidase methods.
Results: Encouraging results prompt that, herbal formulation, which could be proved on excellent sugar level regulator. For efficient phagocytosis by PMNL such as membrane potential, cellular water content, calcium homeostatic, calcium messenger system, contact angle i.e. hydrophobicity measurement and finally particle internalization and Phagocytic index. result were exciting with herbal formulation since it was found to effective in correction of cell parameters related to phagocytosis and remarkable recovery in anti-oxidant enzymes in diabetic patients.
Conclusion: In summary, the results obtained in the present investigation demonstrated\ that the present formulation beneficial in management of diabetic complication.

Keywords: Poly-herbal formulation; hypoglycemic effect; Immune-potentiating; anti-oxidant.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated [1]. Diabetes is a devastating disease throughout the world. It has been estimated that the number of people affected with diabetes in the world will increase to 300 million by 2025. The developed countries such as India, China, and the U.S.A. are presently the countries with the leading number of diabetics [2]. Furthermore, 7% of the residents of the United States are diabetics. Diabetes is the third leading fatal disorder after cancer and heart disease. With diabetes the body cannot regulate the amount of sugar in the blood. Diabetes and its complications take major on the quality of life elderly and the healthcare costs of the society [3].

The most common lipid abnormalities in these patients include hypertriglyceridemia and reduced high-density lipoprotein (HDL) cholesterol levels. As a strong relationship exists between all forms of vascular disease in patients with diabetes and hyperlipidemia. Further, hyperlipidemia is partly responsible for the increased vascular disease as observed in patients with diabetes. Diabetic nephropathy is the leading cause of DM- related morbidity and mortality. The pathogenesis of diabetic nephropathy is related to chronic hyperglycemia and hemodynamic alterations in renal microcirculation and structural changes in glomeruli [4-5]. One of the major complications of diabetes is weakened immune system, which puts the diabetic person at risk for difficult-to-treat and persistent infections and delayed healing of wounds, specially the feet [6]. Many studies have shown that a direct correlationship exists between sugar and free radical formation. Hyperglycemia-induced overproduction of superoxide is the causal link between high glucose and the pathways responsible for hyperglycemic damage. In fact, diabetes is typically associated with increased generation of free radicals and/or impaired antioxidant defense qualifications, representing a central contribution for reactive oxygen species in the onset, progression, and pathological consequences of diabetes. Due to extra generation of free radicals in diabetic condition, the antioxidant status also gets disturbed needing proper attention and care [7-8]. The present study shows that novel polyherbal formulation can be effectively used in the treatment, and management of diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

All the chemicals, drugs and reagents used in this study were of analytical grade.

2.2 Subjects

Subjects were divided Randomized into four groups of 10 subjects in each group. Group I were normal healthy subjects, Group II were diabetics, group III were tolbutamide (oral hypoglycemic drug) treated diabetic patients, group IV were diabetic patients receiving combination herbal formulation in the, dosage of 5 g. /day for 4 weeks. The formulation was administered one hour before food to prevent any interference with its absorption.Diabetic patients were contacted and convinced from two Government Hospitals-Hamidia Hospital, Bhopal, and K. N. Katju Hospital, Bhopal, M. P. (INDIA). The patients were examined at the time of their visit to the outpatient Department (OPD) and consisted of all men (Mean age, 45 ± 12.8 years). No patients had any severe infection nor were on any sort of medication. The volunteers were maintained as per norms of center ethics committee on human research (CECHR) and local ethics committee.

2.3 Plant Materials

Specimens of *Gymnema sylvestre*, *Asparagus racemosus, Withania somnifera, Andrographis paniculata, Tinospora cordifolia* and *Terminalia arjuna* were procured and authenticated from Sanjivani, Department of Forest, Government of Madhya Pradesh, Link Road Bhopal (M.P.).

2.4 Poly-herbal Formulation

Plants shed dried powdered according to Table 1 were mixed in properly. Thereafter the Formulation mixtures were suspended into capsule cell contained 500mg of the blended herbal powder, and preserved till further experimentation.

Table 1. The combination of herbs was follows

S. No.	Botonical Name / Family	Vernacular name	Parts used	Quantity in the Formulat-ion
1.	***Gymnema sylvestre*** (Retz.) R.Br. ex Schult./ ASCLEPIADACEAE	Gudmar	Leaves	100 g.
2.	***Asparagus racemosus*** Willd./ LILIACEAE	Shatavar	Root	100 g.
3.	***Withania somnifera*** (L.) Dunal/ SOLANACEAE	Ashwagandha	Root	50 g.
4.	***Andrographis paniculata*** (Burm. *f.*) Wallich ex Nees / ACANTHACEAE	Kalmegh	Root	100 g.
5.	***Tinospora cordifolia*** (Willd.) Miers. ex Hook. f. & Thoms./ MENISPERMACEAE	Guduchi	Root	100 g
6.	***Terminalia arjuna*** (Roxb. ex DC.) Wight & Arn. / COMBRETACEAE	Arjun, Kahua	Bark	50 g

2.5 Isolation of PMNL (Polymorphonuclear Leukocyte)

PMNL were isolated by the method of Boyum [9] with slight modifications. 10 to 15 ml fasting blood samples were drawn into heparinized tubes already containing 5% dextran (prepared in 0.9% NaCl; use 2 ml 5% dextran for every 10ml of blood). This preparation contained 97-99% PMNL, when viewed under phase contrast microscope (TarLzeif, AXIOVERT-200; NEERI, Nagpur).

2.6 Opsonization of Bacteria

Opsonized bacteria were obtained by the method previously described by Nathan et al. [10] with certain modifications. Overnight cultures *of S. aureus and E. coli* were formalinized (1% v/v), heated at 65ºC for 30 min and then centrifuged at 8000 x g for 15 min. the pellet obtained was washed three times with 0.15 M saline. Two volumes of such a suspension of bacteria were then mixed with one volume of respective antibody and incubated at 37ºC for 30 minutes. Sensitized bacteria thus obtained were centrifuged at 8000 x g for 15 min, washed twice in Hank's balanced salt solution (HBSS) and then re-suspended in HBSS.

2.7 Determination of Blood Glucose Level

The blood glucose level was measured in all the groups by using Glucose oxidase-peroxidase method (Autoanalyser method) [11], Oral glucose tolerance tests: (OGTT) [12].

2.8 Assay of Ouabain Sensitive and Insensitive Adenosine Triphosphatase

PMNL were suspended in 0.34M sucrose and homogenized with chilled teflon pestle for 1 min which yielded greater than 95% rupture. Suspension was spinning at 600 g for 10 min and the resulting pellet was re-suspended in 0.34 M sucrose and used as enzyme source. Membrane ATPase (ouabain sensitive and insensitive) was determined by the method of Post and Sen [13]. The ouabain sensitive ATPase activity was obtained from the difference in total ATPase with ouabain insensitive ATPase activity. The specific activity was expressed as nmol of Pi released/min/mg protein.

2.9 Cellular Water Content

Cellular water content was determined by the method of Parker [14]. Cellular water content was expressed as grm H_2O/kg dry solids.

2.10 Estimation of Cytosolic Calcium Concentration

Cytosolic calcium concentration was assayed by the method of Murphy et al. [15]. The absorbance was measured at the wavelength pair, 675 and 685 nm before and after addition of digitonin. The cytosolic free calcium concentration was assumed to be equal to the free ionic calcium concentration of the medium when no net change of calcium occurred upon addition of digitonin.

2.11 Measurement of Total Cell and Plasma Membrane Calcium Content

Calcium was measured using atomic absorption spectrophotometer (Shandon Southern A3400) by the method of Parker et al. [16]. Readings was taken using an atomic absorption spectrophotometer (Shandon southern A3400) using appropriate standards of $CaCO_3$. Results were expressed as µgs of calcium/mg protein.

2.12 Ca^{2+}/Mg^{2+} - ATPase of the Plasma Membrane

Plasma membrane fraction was prepared as described in methodology (Chari and nath 1984) and was finely suspended in 0.34 M sucrose. The suspension served as the enzyme source. Ca^{2+}/Mg^{2+} - ATPase activity was assayed by the method of Lynch and Cheung [17]. Inorganic phosphate released was measured by the method of Fiske and Subbarow [18] and the blue color developed after 10 min was read at 660 nm. Specific activity was expressed as nmol of Pi liberated/min/mg protein.

2.13 Cyclic-3', 5'-Adenosinemonophosphate Phosphodiesterase Activity

cAMP Phosphodiesterase activity was assayed by the previously described method of Butcher et al. [19]. Specific activity was expressed as nmol of Pi liberated/min/mg protein.

2.14 Phospholipase-c Activity

Phospholipase-c activity using phosphatidylinositol, phosphatidy-1-choline or phosphatidylethanolamine as substrate was assayed by the method described previously by Ottolenghi [20]. After development of the color for 30min at 37ºC. The optical density was read at 740 nm, and specific activity was expressed as nmol of P_i released/min/mg protein.

2.15 Measurement of Contact Angle

Contact angles were measured by the method described previously by Van Oss et al. [21]. The measurement was done by turning the rim of the goniometer until one of the hairs of the cross-hair was tangential to the drop at the place where it was in contact with the surface. The contact angle was then read on the rim of the goniometer. In all cases the angles of ten or more sessile drops of physiological saline were measured.

2.16 Nitroblue Tertrazolium Reduction

Nitroblue tetrazolium reduction index (Phagocytic index) was measured by the method of Baehner and Nathan [22], with slight modifications. The extracts were combined, and the optical density of the purple color of reduced nitroblue tetrazolium (NBT) was determined at 515 nm against a pyridine blank. The optical density of an extract of cells and NBT incubated for 10 sec was also determined, and this value was subtracted from the others as a reagent blank. Resting and phagocytosing values were obtained and the difference (OD per 15 min per 2.5 x 10^6 PMNL) calculated was expressed as phagocytic index.

2.17 Glutathione Peroxidase

Glutathione peroxidase levels were determined by using H_2O_2 as substrate. Paglia and Valentine [23]. The enzyme activity was monitored by decrease in optical density every minute for 10 minutes in a spectrophotometer (Systronics) at 340 nm. Specific activity was expressed as nmole's of NADPH oxidized/min/mg protein.

2.18 Glutathione Reductase

PMNL pellet was suspended in 70% glycerin as suggested by Strauss et al. [24] and glutathione reductase was assayed by the method of Horn [25].

2.19 Superoxide Dismutase

SOD was spectrophotometrically assayed by the method described by Ross et al. [26]. One unit of activity is defined as the amount of enzyme required to inhibit by 50% a standard rate of Ferricytochrome C reduction (units of activity/mg protein).

2.20 Superoxide

The amount of superoxide was measured by the method describedby Johnston et.al. [27].

2.21 Statistical Analysis

All the grouped data were statistically evaluated and the significance of various treatments was calculated using Student's *t*-test. All the results were expressed as mean SD, $P<0.01$, $P<0.001$ was considered significant and highly significant [28].

3. RESULTS AND DISCUSSION

3.1 Results

The present formulations at a prescribed dosage could bring down the sugar level to normal, which was at par with the oral hypoglycemic drug tolbutamide (Table 2). Similar improvement was seen in glucose tolerance studies also(Table 3) in the prescribed dosage of the formulations, the peak value after one hour were normal range (7.77±3.0 mmol/l) and after two hours. The plasma glucose value were not normal, but even lower the initial value (4.52 Vs 4.66 mmol/l) in this case also the result were almost comparable with allopathic drug tolbutamide. Phagocytosis which is the first line of defense gets disturbed in diabetic patients resulting in an array of infections in them. Phagocytosis is carried out by specific group of cells in the body polymorphonuclear leucocyte (PMNL). The activity of ouabain (insensitive) ATPase escalated during phagocytosis in normal PMNL, (control), (91.63 %). PMNL obtained from untreated diabetic patients had quite depressed activity of this enzymes both in resting and phagocytosing state as compared to control (Table 4). Tolbutamide treated diabetic group did not show any significant recovery. The percentage increase being (43.5%) increment in cellular water contents in phagocytosing diabetic PMNL was only (7.2%) PMNL from tolbutamide treated patients' elicited negligible recovery, and the increment in their cellular water content during phagocytosis was (14.48%), encouraging and significant results were observed in the combination drug treated patients. The increase

in cellular water content during phagocytosis was nearly the same as observed in normal PMNL,(40.38%Vs 43.5%). It is evident from the results in Table 5. The leucocyte (Ca^{2+}) of diabetics recorded significant increment (104±0.2 nmol) as compared to control, (67±0.5nmol). The sight, but not insignificant decrease in total as well as plasma membrane (PM), Calcium was noticed in untreated as well as tolbutamide treated diabetic patients. The values of total as well as PM calcium were observed. (Table 6) reveals the cAMP Phosphodiesterase profile of normal diabetic and treated PMNL in resting state. cAMP Phosphodiesterase activity was significantly low in resting diabetic PMNL (1.22±0.09nmol) as compared to control. Fig. 1 represented the fold increase in Phospholipase activity during phagocytosis in normal and pathological groups using PI,PC and PE as substrates fold increase was arrived at by dividing the activity reached during phagocytosis by the respective activities in resting sate. Contact angle of Opsonized E.coli. (29º±1º) and S.aureus (24.3º±1º) were higher as compared to their unopsonized counter parts (26º±1* and 19.7º±1º) respectively. Contact angle was significantly increased in diabetic PMNL (25.2º±0.1º) compared to control (17.20º±0.1º) Phagocytic capability of normal PMNL was higher using Opsonized E.coli (4.6±0.36 particles internalized) S. aureus (22+0.24 particles internalized), as compared to the Phagocytic capability with unopsonized particles (Table 7). Among these E.coli demonstrated a higher Phagocytic capability compared to S. aureus. the Phagocytic index(NBT reduction assay) estimates the ability of neutrophills and macrophages to produce oxygen radicals (O_2,$OH^{-}$$H_2O_2$). The ability of PMNL and macrophages to kill pathogenic microbes is probably one of the most important mechanisms of defense NBT reduction assay (Table 8). It is evident that the Phagocytic index of diabetic PMNL, (0.12±0.05) in highly depressed as compared to normal (0.23±0.02). Table 9 depicts the status of gluthione redox system I diabetic PMNL. Whereas no alteration could be seen in the activity of gluthione reductase, the gluthione peroxidase activity was significantly reduced in diabetic PMNL (46.03±1.12nmol), when compared to control (72.11±4.61nmol), (Fig. 6). The activity of superoxide dismutase was significantly depressed in diabetic PMNL (2.97±0.87 U/mg protein), when compared to normal (5.72±1.19U/mg protein) Table-10. Significantly recovery in superoxide dismutase activity could be visualized in PMNL from herbal formulation treated diabetic patients (4.62±1.26 U/mg protein). Effect of phagocytosis on superoxide dismutase activity was also assessed as depicted in Fig. 2. Superoxide accumulation was significantly enhanced in resting diabetic PMNL (3.16±0.63 nmol), as compared to control PMNL (1.79±0.42 nmol) PMNL from controlled diabetics elicited a partial recovery in superoxide contents (3.04±0.69) in case of TTD and 2.07±0.91 nmol) was case of HFTD, (Table -11). The effect of phagocytosis on superoxide accumulation of normal and diabetic PMNL was depicted in (Fig. 3).

Table 2. Effect of herbal drug formulations on the fasting plasma glucose level in diabetic patients after treatment of four weeks

S.no	Subjects N=10	Fasting plasma glucose (mmol/l) Zero weeks	Four weeks
1	NH	4.46.4±9.5	4.44±5.2
2	DI	9.54±15.4b	15.81±42.6a
3	TTD	9.15±2.0	4.44±1.5
4	HFTD	9.26±28.5a	4.45±2.0

ap<0.0001,bp<0.01

Table 3. Plasma glucose level in glucose tolerance test after four week of treatment

S. no.	Subjects N=10	Plasma glucose(mmol//l)				
		0 hrs	**0.5hrs**	**1hrs**	**1.5hrs**	**2hrs**
1	NH	4.60±6.5	6.13±4.2	8.05±5.3	5.57±6.7	4.78±5.2
2	DI	8.91±14.3a	13.34±77.2b	14.98±90.0b	15.67±81.4a	15.57±83.2a
3	TTD	4.44±1.0	6.09±1.5a	7.95±7.5a	5.46±2.3a	4.41±2.5c
4	HFTD	4.66±2.0	6.21±2.5a	7.77±3.0a	5.81±2.3	4.57±3.6c

ap<0.001,bp<0.001,cp<0.02

Table 4. Change in the activities ouabain sensitive and insensitive ATPase of Polymorphonuclear leucocyte (PMNL)

S. no.	Subject N =10	Na+/K+ - ATPase (ouabain sensitive).mµ moles of Pi liberated/min/mg protein		Na+/K+ - ATPase (ouabain insensitive).mµ moles of Pi liberated/min/mg protein	
		RESTING	PHAGOCYTING	RESTING	PHAGOCYTING
1	NH	4.57±0,41	0.91±0.09	20.93±2.31	40.11±2.31
2	DI	1.39*±0,17	0.69**±0.19	9.64±1.07*	16.83*±2.70
3	TTD	2.01***±0.76	0.81**±0.11	10.23ns±1.91	18.19ns±3.62
4	HFTD	3.95±0,81	0.84±0.2	18.51±1.99	36.11±2.61

**P<0.001 as compared to group-I; **P<0.01 as compared to group-I; ***P<0.02 as compared to group-I; Ns= non-significant as compared to group-I*

Table 5. Change in cellular water contents of PMNL during phagocytosis

S. no	Subject N =10	Water content (Gram water/Kg dry solids)	
		Resting	**Phagocytosis**
1	NH	3.08±0,61	4.42±0.61
2	DI	5.57*±0.51	5.97*±1.02
3	TTD	5.06**± 0.90	5.79**±0.57
4	HFTD	3.12**±0.92	4.38**±0.52

**P<0.001 as compared to group-I; **P<0.02 as compared to group-I*

Table 6. cAMP Phosphodiesterase activity of PMNL in resting state

S.no.	Subject N=10	cAMP Phosphodiesterase activity (nmol PI liberated/min/mg protein)
1	NH	3.51±0.41
2	DI	1.22*±0.09
3	TTD	2.21**±0.43
4	HFTD	3.19ns±0.56

**P<0.001 as compared to group-I; Ns= non significant as compared to group-I*

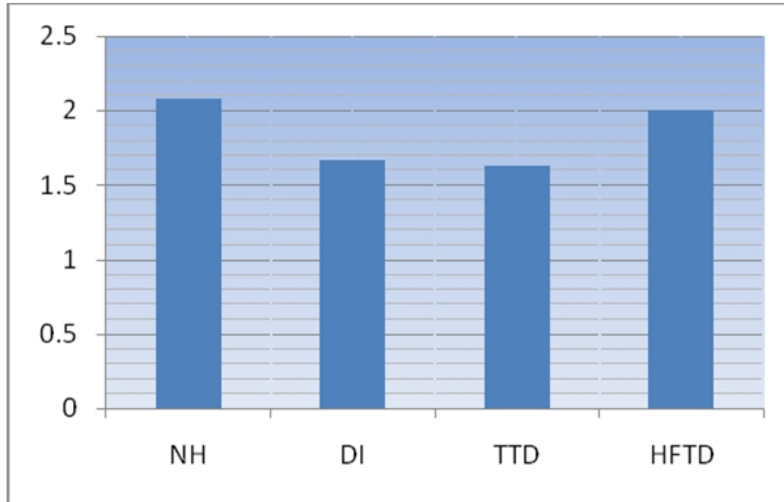

Fig. 1. Effect of Phagocytosis in the alteration of cAMP Phosphodiesterase activity of PMNL

Table 7. Comparative Phagocytic capabilities of PMNL with Opsonized and unopsonized *E. coli* and *S. aureus*

S. no.	Subject N=10	Particles internalized				%PMNL participation in Phagocytosis			
		E.coli		S. aureus		E.coli		S. aureus	
		Opso-nized	Unopso-nized	Opso-nized	Unopso-nized	Opso-nized	Unopso-nized	Opso-nized	Unopso-nized
		Value represented as mean +SE				Only mean values represented			
1	NH	4.6 ±0.36	2.7 ±0.27	2.2 ±0.24	1.3 ±0.12	89	85	51	43
2	DI	1.7 ±0.14*	0.32 ±0.09*	0.21 ±0.03	0.08 ±0.01	64	55	11	11
3	TTD	1.8 ±0.17*	0.34 ±0.21*	0.23 ±0.09*	0.07 ±0.01	65	57	12	12
4	HFTD	4.3 ±0.16**	1.9 ±0.12	1.46 ±0.06*	1.0 ±0.02	79	74	43	39

**P<0.001 as compared to group-I; **P<0.02 as compared to group-I; ***P<0.1 as compared to group-I*

Table 8. Changes in Phagocytic Index (NBT reduction test of PMNL)

S.no	Subjects N=10	Phagocytic index OD=515nm(Phagocytic cells)
1	NH	0.23±0.02
2	DI	0.12±0.05
3	TTD	0.21±0,01
4	HFTD	0.19±0.01

Table 9. Status of glutathione redox system of PMNL under resting condition

S. no	Subject N=10	Gluthione reductase (umol of NADPH oxidized/min/mgprotein)	Glutathione peroxidase (umol of NADPH oxidized/min/mgprotein)
1	NH	2.82±0.58	72.11±4.61
2	DI	2.46±0.41	46.03±1.12
3	TTD	2.49±0.50	57.39±1.17
4	HFTD	2.48±0.42	67-19±1.36

Table 10. Cytosolic superoxide dismutage activity of PMNL under resting condition

S.no	Subject N=10	Superoxide dismutase (cytosolic) U/mg protein
1	NH	5.72±1.19
2	DI	2.97*±0.87
3	TTD	3.01*±1.04
4	HFTD	4.62*±1.26

**P<0.001 as compared to group-I*

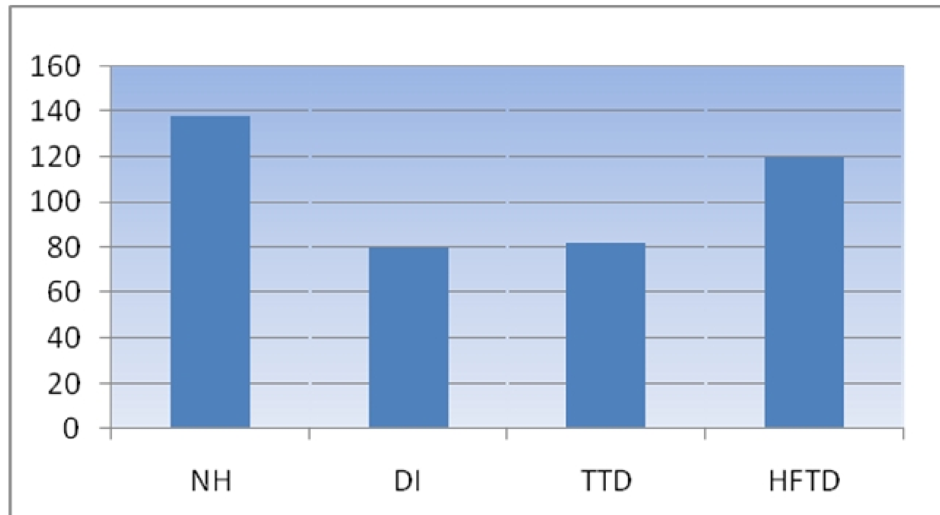

Fig. 2. Effect of phagocytosis in the specific activities of superoxide dismutase

Table 11. Accumulation of superoxide of PMNL under resting condition

S.no	Subject N=10	Superoxide produced (nmol cytochrome-C reduce/10min/mg protein)
1	NH	1.79±0.42
2	DI	3.16*±0.63
3	TTD	3.04*±0.69
4	HFTD	2.07*±0.91

**P<0.001 as compared to group-I*

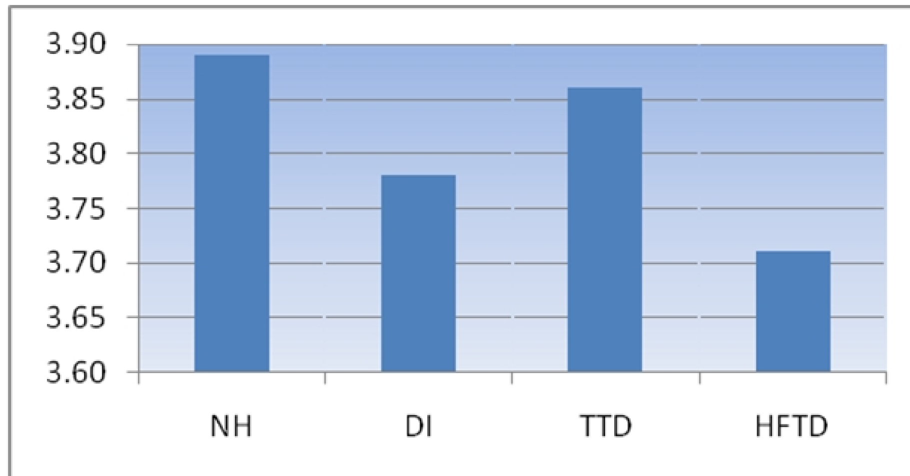

Fig. 3. Effect of phagocytosis in the superoxide accumulation

3.2 Discussion

Many herbal products have been described for the care of diabetes mellitus in ancient literature of 'Ayurveda' in India. The present study demonstional significant improvement in the level of FBG and glucose tolerance with the use of herbal formulation a dosage of 5g/day (containing 1g *Gymonema sylvesterase* in diabetic patients. When used as mono therapy does not causes hypoglycemic and is this termed anti-hyperglycemic [29]. Kar et. al. 2003 investigated that hypoglycemic activity of 24 plants and concluded that *Gymnema sylvestre* was the most potent sugar blocker [30]. Chattopadhyay (1999) studied the comparison of blood sugar lowering activity of *Azadirachta indica*, *Gymnema sylvestre* and several other plants. He found *Azadirachta indica* to have most potent sugar lowering activity followed by *Gymnema sylvestre* [31]. The hypoglycemic action of *Gymnema sylvestre* leaves was first documented in late 1920 [32]. Numerous experimental studies based on clinical observation showed that Phagocytic functions of various cells were decreased in diabetics. Investigations carried out to understand the pathogenesis of these changes in the cholesterol/phospholipid ratio of the cell membrane due to diabetic infection [33] cause hypercholestrolmia [34]. This has been associated with a decreases in membrane fluidity, thus altering several function including the cation transport mechanisms specially Na^+/K^+ ATPase activity [35-36]. A decresase in the activity of Na+/K+ ATPase have been observed in diabetic macrophages and PMNL's. *Androphecous paniculata roots* which were included in the herbal formulation have claimed to be an activator of Na^+/K^+ ATPase activity [37-38]. A recovery in the activity of Na^+/K^+-ATPase in diabetic PMNL consequent to the administration of 5grams/day of the herbal formulation for four weeks also demonstrate significantly recovery in the cellular water contents. Simultaneously activity $Ca^{2+/}Mg^{2+}$ ATPase was also assayed. It is the enzymes responsible for the mobalizaation of calcium from intercellular stores. It is also become imperative to determine the total as well as plasma membrane calcium content. It was successful attempts where the formulation contain Aspargous *reacemosous* and *Tinospora cardifolia* could implement a correction in the cell $[Ca^{2+}]$c. which had been found to be disturb in diabetic mellitus. It is well recognized that in diabetic leucocyte mobility chemotoxins and phagocytosis are grossly decreased [39]. There are no doubt that plats like *Aspargous racemosous* and *Tinospora cordifolia* have been proved to

be immune potentiating as well as antioxidant in the vast literature assayable on medicinal plants. Evidence of opsonisation was affecting a raise in bacterial contact angle, and associated Phagocytic ability with normal PMNL, and the finding of the difference in contact angle (Delta-c) between normal PMNL, with *E. coli*. being greater than *S.aureus* eliciting higher bacterial inter-normalization in proportion to (Delta-c) is in conformation with the concept that hydrophobicity determines Phagocytic capability [40]. Ethanolic extract of *T. cardifolia* has been shown to improve phagoytic function in mice [41]. Root of *A. racemosous*, and *T. cordifolia* are well known for their immune-potentiating effect .But till date no work was ever done on studying the effect of herbal drugs on enzymes, which are so important in maintaining a proper biochemical balance inside the cell. In the present study where roots of above plants were blended and included in the formulation were quite effective since they could demonstratrate a significant recovery by increasing activity of the enzymes Phospholipase-C, in HFTD, PMNL during resting followed by an enhanced increase during phagocytosis. The combination herbal formulation demonstrate a significantly recovery in all the above mentioned parameters. Increment in generation of superoxide anion during phagocytosis is an established fact and in thought to play an important role in microbial killing. It may, however be pointed that superoxide radicals and its product the hydroxyl radical may be very toxic to the host cell as well in order to the inherent toxicity of these free radicals . The cell posses' two strategies quenching system, while the first is superoxide dismutase, which as the name suggest, quenching superoxide [42], the second is glutahione redox system, which among other function degrades peroxides. This may be due to the presence of herbs having antioxidant activity like *Tinospora cardifolia* and *Aspargous racemosous*. Diabetic patients generally are at a higher risk of cardiovascular complication and inflammation , the bark of Arjuna [43], which is cardiac tonic and roots of *withania somnifera* [44], which have an active anti-inflammation activity were added attributed of the herbal formulation.

4. CONCLUSION

The herbal formulations prove its efficacy by reducing higher sugar level, potentiating the immune system and improving the anti-oxidant status of diabetic patients. Present therapeutic strategies mostly try to relief the chemical manifestation of diabetic and its complications. The major challenge in diabetic research is to define not only the cause – effect relationship between various risk factors and complications, but also to compared the effects of pharmaceutical agents that are beneficial in the management of diabetic complications. The specific activity in the formulation has been proven scientifically in the present study and will really prove to be a "**Boon**" for diabetic patients, in the coming future.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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