

Non-Specific Immune Modulation Response by Use of Aboriginal Potent Herbal Combination in Diabetic Urban Population

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Abstract

World, a globally experiencing rapid socioeconomic progress and urbanization, carries a considerable share of the global diabetes burden. In the present study, powdered dodecyl sulphate of 'poly-herbal formulation' was evaluated for its immuno-enhancing/modulation potency to polymorpho nuclear lymphocytes, against normal and diabetic individuals. Subjects were divided into three groups of 140 subjects in each group consisted of all men in urban population (Mean age, 45 + 12.8 years). group I were diabetics, group II were tolbutamide (oral hypoglycemic drug) treated diabetic patients, group III were receiving combination herbal formulation in the, dosage of 5 g. /Day for one month. Results were analyzed using Student 't' test followed by Tukey's test. Significant changes were noticed in Cellular water content, calcium concentration contact angle, Phagocytosis and Phagocytic index, parameters in normal diabetic patients, Tolbutamide treated diabetics and diabetics' individuals treated with different dosages 'polyherbal combination formulation' alone. The efficacy of ' Drug formulation' as an increase phagocytosis activity and strength the non-specific immune system which is the first line of defense in innate immunity gets disturbed in diabetic patients resulting in array of infection in them which might be reduces the risk of non-specific infection of diabetic individuals.

Keywords: Immune-modulation; Hyperglycemia; Phagocytosis; Polymorpho nuclear lymphocytes (PMNL)

Abbreviations: T2DM: Type 2 Diabetes Mellitus; PBMCS: Peripheral Blood Mononuclear Cells; OGTT: Oral Glucose Tolerance Test; NBT: Nitroblue Tetrazolium.

Introduction

Diabetes mellitus is one of the most prevalent and serious diseases in the World. Diabetes mellitus affects

approximately 14% urban and 2.9% rural people and approximately more than 500 million people worldwide and accounts for about one fifth of all expenditures for health care and day by day increase this number rapidly [1-3]. Patients with type 2 diabetes mellitus (T2DM) are immunocompromised and have an increased incidence of infections, mainly in the bone and skin, as well as in the respiratory, enteric and urinary tracts [4]. Diabetic foot ulcers are often complicated by infection and represent the first cause of non-traumatic amputations and are a leading cause of hospitalization among diabetic patients [5]. Diabetes also influences the outcomes of specific infections, such as bacteremia and mortality following community-acquired pneumonia [6]. In addition, it has recently been reported that diabetes triples the risk of hospitalization after influenza A (H1N1) infection and quadruples the risk of intensive care unit admission after hospitalization [7].

The reasons why diabetic patients present an increased susceptibility to frequent and protracted infections are still far from being understood. Several factors have been suggested, including genetic susceptibility to infection, local factors including poor blood supply and nerve damage, alterations in metabolism associated with diabetes, and defects in innate immune defence mechanisms [8]. Regarding the innate immunity, phagocytic activity of peripheral blood mononuclear cells (PBMCs) plays an essential role in protecting the host from infectious diseases, and decreased PBMC function has been implicated in the increased risk of infections that occurs in diabetic patients [9]. Hyperglycemia and poor glycemic control are considered to be the key basal factors in the development of diabetes and its associated secondary complications. Moreover diabetics also suffer from delayed wound healing and depressed infection fighting capability due to decrement in phagocytosis efficiency. Numerous experimental studies based on clinical observation showed that Phagocytic function of various cells were decreased in diabetes, investigation carried out to understand the pathogenesis of these changes have shown that the changes in cholesterol/phospholipids ratio of the cell membrane due to diabetic infection [10,11].

Consequently since phagocytosis is one of the properties of cell membrane. Phagocytosis is carried out by specific group of cells in the body, the efficient working of these cells depends upon their membrane potential regulated the enzymes Na^+/K^+ , ATPase and calcium homeostasis in their intracellular milieu [12]. Diabetics

are more prone to long lasting infections due to several above reasons. The modulation of immune response by using medicinal plant products as a possible therapeutic measure has become a subject of active scientific investigation. The basic concept has, however, existed in the ancient Vedic scripture, "The Ayurveda. The main reason for the sages request to the gods was to avoid diseases and remain fit for a long time. Hence the major approach to therapy in Ayurveda is one which emphasizes prevention. Certain medicinal plants can play a great role in improving phagocytosis thereby potentiating the immune system the presented study was to evaluate the efficacy of novel herbal drug formulation to enhance the non-specific immune system from diabetic patients.

Materials & Methods

The plants were selected on the bases of their potential activities and wide medicinal uses in the traditional literatures. The ease of availability of plant is also taken into consideration during selection. Roots of *Asparagus racemosus* (Shatavari), *Andrographis paniculata* (Kalmegh), and *Tinospora cordifolia* (Guduchi) rhizomes and leaves of *Gymnema sylvestre* (Gudmar) were collected and authenticated by the "Sanjivani Nursery", Department of Forest, Government of Madhya Pradesh, Link Road, Bhopal (M.P.) India [8-11].

Formulation

After preparation of dry powder and mixed properly until a homogeneous mixture was obtained, the next step was to formulate a polyherbal preparation. The mixture was encapsulated to ensure blinding, uniformity of administration and for convenience of patients (Table 1). One capsule contained 500mg of the blended herbal powder [12].

S. No	Subjects N=60	Fasting Plasma Glucose(mg/dl)	
		Zero Weeks	Four Weeks
1	NH	78.4±8.5	79.0±5.2
2	DI	174±15.4b	288.6±42.6a
3	TTD	163.9±2.0	82..1±1.5
4	HFTD	165.9±28.5a	80.1±2.0
ap<0.0001,bp<0.01			

Table 1: Effect of combination herbal drug formulations on the fasting plasma glucose level in diabetic patients after treatment for four weeks.

Ethics Statement

Informed written consent was obtained from all participants and the study was approved by the hospital's human ethics committee (K.N.Katju Hospital and Hamidia Hospital). After taking permission for clinical studies from the volunteers were selected as per norms of center ethics committee on human research (CECHR) and local ethics committee. (Reg.No.1/441). No patients had any severe infection nor were on any sort of medication.

Case-Control Study

In this study we have investigated the effect of type 2 diabetes and the degree of glycaemic control on the phagocytic activity of monocytes/macrophages. On this basis, of case control study, a total of 60 type-2 diabetic patients of urban origin and free of chronic diabetic complications who were attending the outpatient Diabetes Unit of hospital were recruited for the study over a 1-month period (cases). Subjects were divided into four groups of 60 subjects. Group I (NH) were normal healthy subjects, Group II (DI) were diabetics, group III (TTD) were tolbutamide, group IV (HFTD-1, HFTD-2, HFTD-3 (three different dosage class) were diabetic patients receiving combination herbal formulation in the, dosage of 1g/day, 2.5g/day and 5 g./day for 4 weeks [13]. 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.

Determination of Blood Glucose Level

The blood glucose level was measured in all the groups by using Glucose oxidase-peroxidase method (Autoanalyser method), Oral glucose tolerance test: (OGTT) [14].

Isolation of PMNL (polymorphonuclear leukocyte)

After an overnight fast of 10 h, venous blood was collected from the antecubital vein in preheparinized syringes at 8.00 am. During the next 60 minutes 100 μ L of the heparinized whole blood was exposed for 30 minutes at 37°C in the dark to 20 μ L of eppendorf tube. PMNL were isolated by the method of with slight modifications [15]. 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. (Tarl-zeif, AXIOVERT-200).

Ca²⁺/Mg²⁺ - 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 [16]. The suspension served as the enzyme source. Ca²⁺/Mg²⁺ - ATPase activity was assayed by the method of Lynch and Cheung [17]. Incubation mixture consisted of 40mM Tris-HCl (pH 7.4), 3mM MgCl₂, 80mM NaCl, 20mM KCl, 0.2mM Ouabain, 2mM ATP, 100 μ M Ca²⁺ and enzyme preparation in a final volume of 0.4 ml. Reaction mixture was incubated for 30 min at 37°C. Reaction was terminated by the addition of 0.08 ml of 5% solution of sodium dodecyl sulphate. Inorganic phosphate released was measured and the blue color developed after 10 min was read at 660 nm. Specific activity was expressed as n mol of Pi liberated/min/mg protein [18].

Cyclic-3', 5'-Adenosinemonophosphate Phosphodiesterase Activity

PMNL were suspended in 0.34 M sucrose and homogenized with chilled Teflon pestle for 1 min. Cells were further disrupted by sonication for 1 min in a Ralsonics Sonicator with controls set at 140. Broken cells were centrifuged at 600 x g for 10 min and the supernatant so obtained was again centrifuged at 15,000 x g for 20 mins. The supernatant so obtained served as the enzyme source. cAMP phosphodiesterase activity was assayed by the previously described method of Butcher, et al. [19].

The reaction mixture consisted of 0.36 μ moles cAMP, 1.8 μ moles MgSO₄, 36 μ moles Tris-HCl buffer (pH 7.5) with a suitable dilution of the enzyme source in a final volume of 0.9 ml. This mixture was then incubated at 30°C for 30 min. After the first 20 min. of incubation, 0.1 ml Crotalus Atox venom solution was added containing 0.1 mg venom in 1 x 10⁻²M Tris-HCl buffer (pH 7.5). The entire reaction was terminated by the addition of 0.1 ml of cold 55% TCA. The precipitate was removed by centrifugation and aliquot of the supernatant was analyzed for inorganic phosphate by the modified method of Fiske and Subbarow. Specific activity was expressed as nmol of pi liberated/min/mg protein [20].

Phospholipase Activity

Broken PMNL were centrifuged at 600 x g for 10 min. and the pellet thus obtained was washed twice and resuspended in 0.34 M sucrose. This suspension served as the enzyme source. Phospholipase c activity using phosphatidylinositol or phosphatidyl choline or phosphatidylethanolamine as substrate was assayed by the method described previously by Ottolenghi [21]. Required quantity of phosphatidyl-inositol, phosphatidyl choline and phosphatidyl-ethanolamine was suspended in triple distilled water and sonicated vigorously for 20 min for a complete dispersion of aforementioned phospholipids. Reaction mixture consisted of 5 µmol sonicated phosphatidylinositol or phosphatidylcholine or phosphatidylethanolamine, the desired enzyme aliquot, sufficient water and Tris-maleate buffer (pH 7.2) to make the final 5ml system, 5mM with respect to tris.

The mixture was then incubated at 37°C and duplicate 2ml samples were taken at appropriate times and added to centrifuge tubes containing 4ml cold 10% HClO₄, 0.5 ml 2% albumin was then added to aid precipitation. The tubes were then allowed to stand in ice for 30 min and then centrifuged. The remaining floating particles were filtered off through a Millipore filter. Digestion of the filtrate was carried out in an oil bath at approximately 170°C for 2 to 2.5 hrs. The solution was then allowed to cool. Phosphate measurement was done by addition of 10ml of the reagent used by Lowry (50 µl of 0.25% ammonium molybdate in 0.1 N sodium acetate to which add 1ml of 10% ascorbate per 10ml solution) [22]. After development of the color for 30min at 37°C, the optical density was read at 740 nm Specific activity was expressed as nmol of P_i released/min/mg protein.

Phagocytosis

Experiments on phagocytosis, unless otherwise stated, were performed by suspending PMNL in Hank's balanced salt solution (with glucose) and incubating with 0.01 ml polystyrene latex beads for 20 minutes at 37° C in a shaking water-bath. Phagocytosis was stopped by the addition of one mg iodoacetate a preparation of zymosan granules (0.01ml) was used sometimes in place of latex beads [23].

Measurement of Phagocytic Index

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 10⁶ PMNL) calculated was expressed as phagocytic index [24].

Statistical Analysis

Normal distribution of the variables was evaluated using the Kolmogorov-Smirnov test. Data were expressed as the means and standard deviation. Comparisons between groups were made using the Student's *t* test for continuous variables and the χ^2 test for categorical variables. All p values were based on a two-sided test of statistical significance. Significance was accepted at the level of p<0.05. Statistical analyses were performed using the SPSS statistical package (SPSS, Chicago, IL, USA [25]).

Results

Many herbal products have been described for the care of diabetes mellitus in ancient literature of 'Ayurveda' in India. The present study demonstrated significant improvement in the level of FBG and glucose tolerance with the use of herbal formulation a dosage of 5g/day (containing 1g *Gymnema sylvestre* in diabetic patients when used as mono therapy does not cause hypoglycemic and is termed anti-hyperglycemic. 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 1). Similar improvement was seen in glucose tolerance studies also Table 2 in the prescribed dosage of the formulations, the peak value after one hour were normal range (140.3±3.0 mg/dl) and after two hours the plasma glucose value were not normal, but even lower the initial value (82.5 Vs 84.1 mg /dl) in this case also the result were almost comparable with allopathic drug tolbutamide.

S. No	Subjects N=60	Plasma Glucose (mg//dl)				
		0 hrs	0.5hrs	1hrs	1.5hrs	2hrs
1	NH	81.0±6.5	112.5±4.2	142.2±5.3	98.5±6.7	84.3±5.2
2	DI	160.7±14.3a	240.5±77.2b	270.0±90.0b	282.5±81.4a	273.1±83.2a
3	TTD	80.0±1.0	109.0±1.5a	143.3±7.5a	98.5±2.3a	79.5±2.5c
4	HFTD	84.1±2.0	112.0±2.5a	140±3.0a	104.7±2.3	82.5±3.6c

ap<0.001,bp<0.001,cp<0.02

Table 2: Plasma glucose level in glucose tolerance test after weeks of treatment.

S. No	Subject N =60	Na+/K+ - ATPase (ouabain sensitive). μ m moles of Pi liberated/min/mg protein		Na+/K+ - ATPase (ouabain insensitive). μ m moles of Pi liberated/min/mg protein	
		Resting	Phagocytosing	Resting	Phagocytosing
1	NH	4.67±0.41	0.94±0.09	19.93±2.31	42.11±2.31
2	DI	1.37*±0.17	0.69**±0.19	9.64±1.07*	16.83*±2.70
3	TTD	2.11***±0.76	0.81**±0.11	10.23ns±1.91	18.19ns±3.62
4	HFTD	3.93±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 3: change in the activities ouabain sensitive) and insensitive ATPase of Polymorphonuclear leucocyte (PMNL).

Phagocytosis which is the first line of defiance 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 normal, (control), Table 4 tolbutamide treated diabetic group did not show any significant recovery As shown (Figure 1), the leucocyte (Ca²⁺) of diabetics recorded significant increment (104±0.2 nmol) as compared to normal (control), (67±0.5nmol),(p<0.001). 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 (Figure 2).

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S. No	Subject N=60	cAMP Phosphodiesterase activity (nmol PI liberated/min/mg protein)
		1
2	DI	1.32*±0.09
3	TTD	2.26**±0.43
4	HFTD	3.18ns±0.56

*P<0.001 as compared to group-I

Ns= non significant as compared to group-I

Table 4: cAMP Phosphodiesterase activity of PMNL in resting state.

S.No	Subject N=60	Phospholipase-c (n mol of Pi liberated/ min/mg protein)		
		substrates		
		Phosphatidylinositol	phosphatidylcholin	Phosphatidylethanolamine
1	NH	3.76±0.16	5.14±0.64	4.73±0.35
2	DI	1.43*±0.32	2.41*±0.43	2.13*±0.11
3	TTD	1.91*±0.34	3.21*±0.72	2.98*±0.71
4	HFTD	3.01*±0.27	4.96*±0.2	4.39**±1.99

*P<0.001 as compared to group-I

*P<0.02 as compared to group-I

Table 5: Phospholipase-c activity of PMNL using varied substrate under resting condition.

Table 4 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

normal (control). Figure 3 represented the fold increase in activity of cAMP Phosphodiesterase of normal diabetic, tolbutamide treated and herbal drug treated PMNL during phagocytosis. The activity of this enzyme rises 2.08 fold in normal phagocytosing PMNL as compared to resting values. The raise was only 1.63 fold in diabetic PMNL. There was a partial recovery in the fold increase of

phagocytosis PMNL of controlled diabetic patients, Response with tolbutamide being quite insignificant (1.6 fold) and herbal formulation being appreciable 2.0 fold (Table 5). Contact angle of Opsonized E.coli. ($29^{\circ} \pm 1^{\circ}$) and S.aureus ($24.3^{\circ} \pm 1^{\circ}$) were higher as compared to their unopsonized counter parts ($26^{\circ} \pm 1^{\circ}$ and $19.7^{\circ} \pm 1^{\circ}$ respectively).

S.No	Subject	Particles internalized				%PMNL participation in Phagocytosis			
	N=60	E.coli		S. aureus		E.coli		S. aureus	
		opsonized	unopsonized	opsonized	unopsonized	opsonized	unopsonized	opsonized	unopsonized
		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	88	85	51	43
2	DI	1.7±0.14*	0.32±0.09*	0.21±0.03	0.08±0.01	68	55	11	11
3	TTD	1.8±0.17*	0.34±0.21*	0.23±0.09*	0.07±0.01	63	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 6: comparative Phagocytic capabilities of PMNL with Opsonized and unopsonized E.coli and S. aureus.

S.No	Subjects	Phagocytic index
	N=60	OD=515nm(Phagocytic cells)
1	NH	0.26±0.02
2	DI	0.14±0.05
3	TTD	0.23±0.01
4	HFTD	0.22±0.01

Table 7: Changes in Phagocytic index (NBT reduction test of PMNL).

Contact angle was significantly increased in diabetic PMNL ($25.2^{\circ} \pm 0.1^{\circ}$) compared to normal ($17.20^{\circ} \pm 0.1^{\circ}$) (Figure 4). Phagocytic capability of normal PMNL was higher using Opsonized E.coli (4.6±0.36 particles internalized) S. aureus (2.2±0.24 particles internalized), as compared to the Phagocytic capability with unopsonized particles (Table 6). Among these E.coli demonstrated a higher Phagocytic capability compared to S. aureus. The Phagocytic index (NBT reduction assay) estimates the ability of neutrophils 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 7). It is evident that the Phagocytic index of diabetic PMNL, (0.12±0.05) is highly depressed as compared to normal (0.23±0.02).

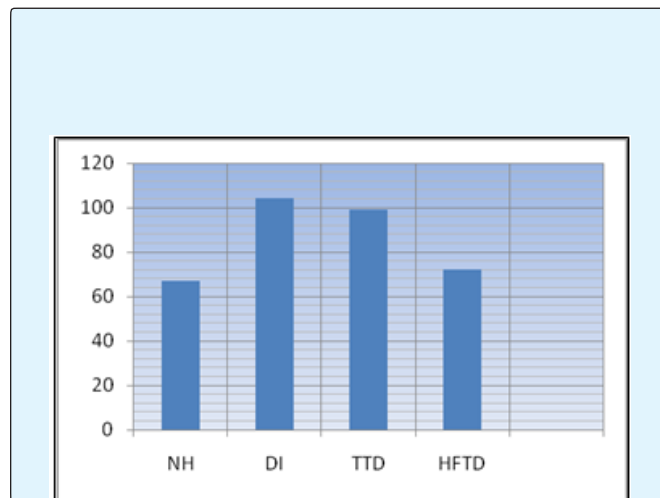


Figure 1: Alteration in cytosolic calcium concentration [Ca²⁺] of Polymorphonuclear leucocyte (PMNL).

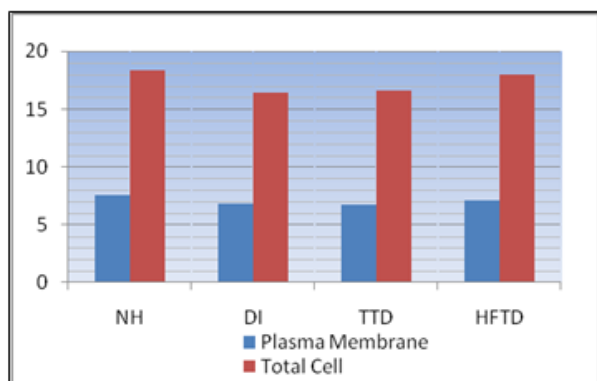


Figure 2: Total Cell and Plasma membrane Calcium content of Polymorphonuclear leucocyte (PMNL).

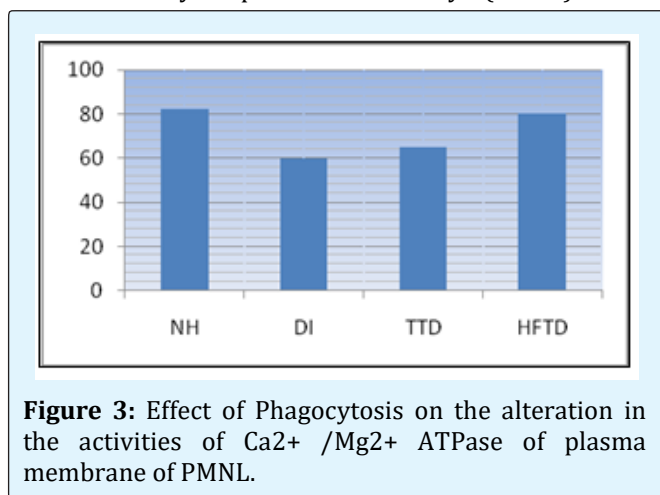


Figure 3: Effect of Phagocytosis on the alteration in the activities of Ca^{2+} /Mg^{2+} ATPase of plasma membrane of PMNL.

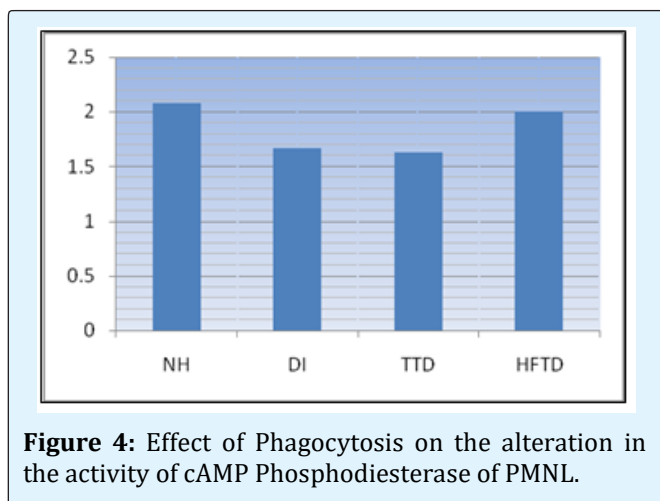


Figure 4: Effect of Phagocytosis on the alteration in the activity of cAMP Phosphodiesterase of PMNL.

Discussion

In the present study we provide evidence that in diabetic mellitus (T2DM) patients the attenuated phagocytic activity of PMNLs exhibits a significant reversibility after glycemic control is improved. In addition, our baseline results confirm and extend previous reports concerning the impairment of phagocytosis that occurs in PMNLs from diabetic patients [26]. Previous studies have described diminished PMNLs function (i.e. defective chemotaxis, bacterial killing, superoxide production, leukotriene release, lysosomal-enzyme secretion and endoplasmic reticulum stress) as well as altered basal levels of intracellular calcium and superoxide in diabetes [27].

In the present study a homogenous population of patients with T2DM was analyzed, the number of subjects included was sufficient for the purpose of the study, a close matched control group was included, the collection of PMBCs was accurate and the method used for measuring phagocytic activity was highly sensitive and reproducible [28]. Nevertheless, further studies addressed to examining the clinical usefulness of this method for evaluating the risk of diabetic patients to acquire infections are needed. Phagocytic cells are the cornerstone of the innate immune system, and individuals with inherited defects in phagocyte function typically present recurrent, unusual, and/or difficult to clear bacterial infections [29]. Our findings reinforce the theory that, in contrast to the sustained immune deficits that occur in genetic disorders, the extent of impairment of phagocytosis in diabetic patients oscillates in relation to glycemic control [30].

Therefore, persistently poor glycemic control could have a progressively deleterious effect, predisposing affected individuals to an increased incidence and severity of infection [31,32]. In addition, our results suggest that an improvement of phagocytic activity can be considered as another beneficial effect of hyperglycemia optimization. The reasons why hyperglycemia or related pathways down regulate phagocytic activity remain to be elucidated [33]. The deleterious effects of free radicals are enhanced in diabetes, because they are less easily eliminated as a result of the reduced effectiveness of the protecting agents. Diabetes activates aldose-reductase which leads to a reduction of glucose excess in sorbitol (polyol pathway) [34].

This pathway is a great consumer of NADPH, which is then less available for PMNLs functions and for free radical elimination by antioxidants. In fact, aldose reductase inhibition enhances neutrophil oxidative killing

by diabetic neutrophils. Although our data on the improvement of phagocytosis dysfunction [35]. 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 cause hypercholesterolemia [36]. This has been associated with a decrease in membrane fluidity, thus altering several functions including the cation transport mechanisms specially Na^+/K^+ ATPase activity.

A decrease in the activity of Na^+/K^+ ATPase has been observed in diabetic macrophages and PMNL's. *Andropogon paniculata* roots which were included in the herbal formulation have claimed to be an activator of Na^+/K^+ ATPase activity. 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 $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase was also assayed. It is the enzymes responsible for the mobilization 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 containing *Asparagus racemosus* and *Tinospora cordifolia* could implement a correction in the cell $[\text{Ca}^{2+}]_c$ which had been found to be disturbed in diabetic mellitus. It is well recognized that in diabetic leucocyte mobility chemotaxis and phagocytosis are grossly decreased [37]. There are no doubt that plants like *Asparagus racemosus* 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 affecting a raise in bacterial contact angle and associated phagocytic ability with normal PMNL, and the finding of the difference in contact angle ($\Delta\theta$) between normal PMNL, with *E.coli* being greater than *S.aureus* eliciting higher bacterial internalization in proportion to ($\Delta\theta$) is in conformation with the concept that hydrophobicity determines Phagocytic capability. Ethanolic extract of *T. cordifolia* has been shown to improve phagocytic function in mice [38]. Root of *A. racemosus*, and *T. cordifolia* are well known for their immune-potentiating effect [39,40].

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 demonstrate a significant recovery by increasing activity of the enzymes Phospholipase-C, in HFTD, PMNL during resting followed by an enhanced increase during phagocytosis [41]. The combination herbal formulation demonstrate a significant 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 null the inherent toxicity of these free radicals [42-45].

Conclusion

The herbal formulation could thus prove its efficacy by reducing higher sugar level; potentiating the immune system and improving the anti-oxidant status of diabetic patients the presence of T2DM and the degree of glycaemic control are related to substantially impaired phagocytosis activity which is reversible after glycaemic control has been improved. Our results suggest that an improvement in phagocytic activity can be added to the beneficial effects of metabolic optimization. Further studies addressed to examining whether this improvement of phagocytic activity leads to a reduction of the rate of infection in diabetic patients are needed. Present therapeutic strategies mostly try to relieve 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 compare 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.

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