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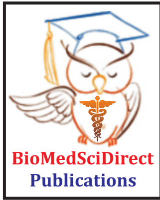

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Original Article

Supplementation with a polyherbal extract and melatonin together with exercise effectively reverses diabetic glycaemic status and carbohydrate metabolism and insulin level.

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A R T I C L E I N F O

Keywords:
Poly herbal extract
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A B S T R A C T

In the present study, a multi-dimensional therapeutic approach for combating experimentally induced Type I diabetes using polyherbal therapy with melatonin supplementation and an exercise regimen (S+M+PE) is employed in both diabetic (DC) and non diabetic (NC) animals. Adult albino rats were made diabetic by a single intraperitoneal injection of alloxan (120 mg / kg body weight). After a duration of 7 days, animals with a blood glucose level of 300 mg / dl or higher were considered diabetic. Control and experimental animals were subjected to swimming exercise together with administration of melatonin and PE for a further duration of 15 days and, upon sacrifice, various parameters related to glycaemic status and carbohydrate metabolism were evaluated. Results revealed a much better glycaemic regulation and carbohydrate metabolism. The glucose clearance rates of DC.S+M+PE animals under both glucose challenge and exogenous insulin were much greater than both NC and DC animals. Observed increase in insulin levels in both NC and DC animals subjected to S+M+PE was an indicative of observed near normal glucoregulation with combination therapy. S+M+PE exerted favourable influence on glucose uptake and utilization by way of increased insulin titre and sensitivity as well as up regulation of GLUT-4. In conclusion it can be said that S+M+PE is an effective combination therapy exerting effective glucoregulation and correction of carbohydrate metabolism.

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1. Introduction

The incidence of diabetes is on the increase and is largely related to the prevalence of obesity and sedentary lifestyle. Joslin [1] was the first one to describe about the significance of exercise in the effective management of diabetic symptoms and his idea of managing blood glucose levels to keep away all secondary complications was very successful. According to American Diabetes Association, exercise is an important tool for the treatment of diabetes [2]. Insulin action of entire body in an individual improves if undergoing proper exercise [3, 4], especially in skeletal muscle, which is the major site for insulin stimulated glucose uptake. This may be mediated via the increase in GLUT-4 level and/or translocation, improving insulin-stimulated glucose transport in skeletal muscle [5, 6].

Just as exercise is an advised regimen for management of diabetes, herbal preparations are also being studied increasingly for their antihyperglycaemic properties. Indian system of medicine is one of the oldest and there are more than 100 medicinal plants mentioned in this system including the folklore medicines for the treatment of diabetic complications; theses mentioned plants are active at their best either individually or in combination [7]. India has a very long and safe history of usage of many herbal drugs as, there are officially recognized systems of health care like Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy as, since more than 500,000 non allopathic practitioners are being trained in more than 400 medical colleges across the country. Apparently, this system of therapy can no longer be considered as traditional or folklore herbal practices [8]. These are to be considered as basic axioms which are leading towards a logical and systemic approach of pathogenesis and diagnosis [9].

Melatonin is another candidate natural molecule of the body considered in the present study as an antidiabetic agent. Several studies have shown the protective effect of melatonin against streptozotocin induced pancreatic β cell damage and the subsequent development of Type I Diabetes [10, 11, 12, 13, 14, 15]. Long-term melatonin administration has also been shown to reduce hyperlipidemia and hyperinsulinemia, and restore altereratios of polyunsaturated fatty acids in serum and tissues of diabetic rats [16]. Treatment with insulin and melatonin

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suppress hyperglycemia, prevent oxidative damage and restore completely endothelial function in the aorta and corpus cavernosum of diabetic rats [17].

In view of the above reports and, in continuation of our previous studies that have dealt with a combination treatment of exercise and polyherbal extract in Type I diabetic rats , the present study has been designed keeping in view the glucoregulatory potentials of both exercise and extract. The present treatment deals with a combination of melatonin administration along with exercise and polyherbal extract treatment aimed at a multidimentional approach to target the multifaceted manifestations of diabetes.

2. Materials and Methods

2.1.Details of plants selected for the study

Seeds of *Cassia fistula* (Fabaceae), and, leaves of *Langerstromia flos reginee* (Lythraceae), *Murraya koenigii* (Rutaceae), *Annona squamosa* (Annonaceae), *Ocimum sanctum* (Lamiaceae), *Coccinia indica* (Cucurbitaceae) and *Mangifera indica* (Anacardiaceae) were used for the preparation of a polyherbal extract. The plant material after collection was identified by Prof. M. Daniel (Head, Department of Botany, M.S.University of Baroda, Vadodara).

2.2.Preparation of polyherbal extract (PE)

Equal amount (250 grams) of fresh leaves/seeds was plucked and separated from the twigs. Leaves were chopped into small pieces and shade dried and then ground in a mixer along with the seeds of *Cassia fistula* which were dried separately to get a powder mixture. The powder was extracted with distilled water using a soxhelt apparatus at boiling temperature (100^0C) up to 10 h; a dark brown coloured extract was obtained. This dark brown extract was cooled and filtered to remove the residue. The extract was concentrated on a rotavapour under reduced pressure and then dried to get a powder. The dried powder was diluted with saline in required proportion for the study. The PE was administered to animals orally using oral gavage.

2.3.Swimming protocol for exercise

Animals were subjected to swimming exercise and were made to swim in a tank with a dimension (150X90X70) (length X breath X height), filled with water to a depth of 30-45 cm, once per day between 08:30 and 9:00 hrs. Animals were acclimatized by making them to swim for 5 days prior to the commencement of the experimental schedule. The acclimatized animals were divided into different experimental groups and were subjected to swimming exercise for 15 days for 30min [18, 19].

2.4.Experimental animals

Female Wistar rats (200-250 g) were housed in the departmental animal house under controlled room temperature (21 ± 2 ºC). The animals were provided with rat chow and water ad libitum. The rat chow was purchased from M/s Pranav Agro Ltd., Baroda. The experiments were carried out after the approval of Animal Ethical Committee of Department of Zoology, The M.S. University of Baroda, Vadodara (Approval No. 827/ac/04/ CPCSEA), and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines were followed strictly. Diabetes was induced in experimental rats by single intraperitonial injection of alloxan (120 mg/kg body weight) as per the procedure described elsewhere [18, 19]. Animals having fasting blood glucose levels higher than 300 mg/dl were used for the experiments.

2.5.Experimental design

Rats were divided into four groups of six rats each.

Group I: (NC) Control rats treated with saline as vehicle for 15 days.

Group II: (NC+PE+S+M) Non diabetic rats subjected to swimming exercise for a period 15 days along with administration of polyherbal extract at a dose of 250mg/kg body weight at 8:00 hrs and melatonin at a dose of 1mg/kg body weight at 18:00hrs daily for a period of 15 days.

Group III: (DC) Diabetic rats treated with saline as vehicle.

Groups IV: (DC+PE+S+M) Diabetic rats subjected to a combination of Swimming exercise and administration of PE at a dose of 250mg/kg body weight along with melatonin at a dose of 1mg/kg body weight at 18:00hrs daily, for a period of 15 days after the induction of diabetes.

2.6.Biochemical analysis

At the end of 15 day treatment schedule, the rats were sacrificed by cervical dislocation after an overnight fast. Liver, Muscle and Kidney were excised out and stored at -80^0C for further analysis. Serum glucose (Agappe Diagnostics kit), and Insulin (Rat Insulin ELISA kit from MERCODIA, Sweden), were assayed using the above kits. Protein was estimated by Lowry et al., [20], glycogen by Seifter et al, [21], glycogen phoshphorylase by Cahill [22] and Glucose-6-phosphatase by Harper [23].

2.7.Western Blot: GLUT 4 expression in muscle tissue

Cytosolic fractions were prepared from skeletal muscles (gastrocnemius) from both control and test animals as described by Dombrowski et al [24]. Briefly stated, 100 mg of muscle was homogenized in an ice-cold homogenization buffer (1:10 w/v) containing 25 mmol 1^{-1} 4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid (HEPES), 20mMb^{-1} glycerophosphate, 2 mmol 1^{-1} ethylenediaminetetraacetic acid (EDTA), 250 mmol 1^{-1} sucrose, 3.3 mg 1^{-1} leupeptin, 3.3 mg 1^{-1} aprotinin, 100 mg 1^{-1} trypsin inhibitor and 1 mmol 1^{-1} PMSF at pH 7.4 using a polytron-equipped homogenizer at a precise low setting on ice. The resulting homogenate was centrifuged at 1300Xg for 10 min at 40^0C. The supernatant was saved and the pellet was resuspended in a homogenization buffer and again spun at 1300Xg for 10 min at 40^0C and, the supernatant of this spin was combined with the first one and again spun at 9000Xg for 10 min at 40^0C. The resulting supernatant was further centrifuged at 1,90,000Xg for 1 h (Preparative Ultracentrifuge, Hitachi, Japan). The resultant supernatant was saved, and sampled as a cytosolic fraction for GLUT4 protein analysis. Protein concentration was further determined by the method of Lowry et al [20].

2.8.Separation of Proteins

Briefly, each sample (25 mg) was subjected to heat denaturation at 96^0C for 5 min with Laemmli buffer. The proteins were resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) on 10% polyacrylamide gels as described by Laemmli [25] and then electrophoretically transferred to polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences, UK). The membrane was blocked with phosphate-buffered saline plus 0.3% Tween-20 (PBST) containing 10% non-fat dry milk for 2 h and then incubated with antiGLUT-4 (1:2000; Santa Cruz Biotechnology, USA), and b-actin (1:2000; Santa Cruz Biotechnology, USA) primary antibodies overnight. After three washes with PBST, the membrane was re-blocked and incubated with secondary antibody (horseradish peroxidaseconjugated donkey anti-rabbit IgG; 1:5000; Sigma, St. Louis, MO, USA) for 2 h at

room temperature. The blots were then rinsed in Tris buffered saline with 0.05% Tween 20 and immunoreactive bands were detected by the Enhanced Chemiluminescence Reagent (ECL; Amersham Biosciences, UK). Images were captured with a ChemiDoc TM XRS system (Bio-Rad Laboratories, CA, USA). Later, the membranes were incubated in stripping buffer (50 ml containing 62.5 mmol 1^{-1} TrisHCl (pH: 6.8), 1 g SDS and 0.34 ml -β mercaptoethanol) at 550C for 40 min. After this, the membrane was reprobed using a β-actin antibody (1:2000). All protein bands were quantified (using Quantity one software system, Bio-Rad) and normalized against internal control β-actin.

2.9.Oral Glucose Tolerance Test (OGTT)

At the end of treatment schedule, animals were fasted overnight and glucose tolerance test was done by feeding them orally with a glucose solution at a dose of 2g/kg body weight. Blood was collected from the retro orbital sinus at 0,30,60,90 and 120min after glucose load. Serum was separated and, glucose was estimated in all the collected samples to get a tolerance curve for all the experimental groups [18, 19].

2.10.Insulin Response Test (IRT)

Response to insulin was checked by injecting Insulin to the rats at a dose of 1U/kg body weight intraperitoneally (i.p) in the fed state, a day following the completion of treatment and blood was collected at 0, 30, 60, 90, and 120 min from the retro orbital sinus under mild ether anesthesia . Serum was separated and used to estimate glucose level and, an insulin response curve was drawn to evaluate the results [18, 19].

2.11.Statistical analysis

Statistical evaluation of the data was done by one way ANOVA followed by Bonferroni's Multiple comparison test. The results are expressed as mean ± S.E.M using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA/USA.

3.Results

3.1. Glycaemic status and insulin levels (Table 1)

Table 1. Levels of fasting and fed serum glucose (mg/dl) and Insulin μg/L in exercised and extract and melatonin treated non diabetic and diabetic rats

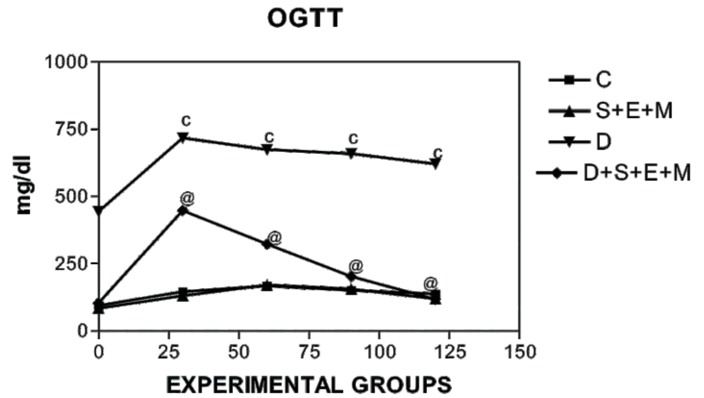
Groups	Serum glucose Fasting	Serum glucose Fed	Insulin(μg/l)
NC	93.97±3.5	113.36±3.18	0.34±0.01
NC+S+PE+M	83.66±5.49	120.6±1.45[b]	0.42±0.017[c]
DC	443.3331.21[e]	655.337.69[e]	0.16±0.012[c]
DC+ S+PE+M	103.33±7.22[@]	176±6.66[@]	0.31±0.01[@]

Data are expressed as Mean±SE
NC = Non - diabetic control, NC+S+E+M = non-diabetic control+polyherbal extract+swimming+melatonin
DC= diabetic control, DC+S+E+M = diabetic control+ swimming+polyherbal extract+ melatonin. b)p<0.025,
c) p<0.01, e) p< 0.0005 compared to NC and @) p< 0.01 compared to DC

Non-diabetic animals subjected to combinational therapy of S+PE+M showed hypoglycaemia in the fasted state and hyperglycemia in the fed state. There was significant anti-hyperglycaemic effect of the treatment as the glucose level in fasted and fed states was close to normoglycaemia. Insulin titre was significantly low in DC animals and, combinational therapy increased the insulin titre in NC animals and remained in the normal range, though slightly lower, in DC animals.

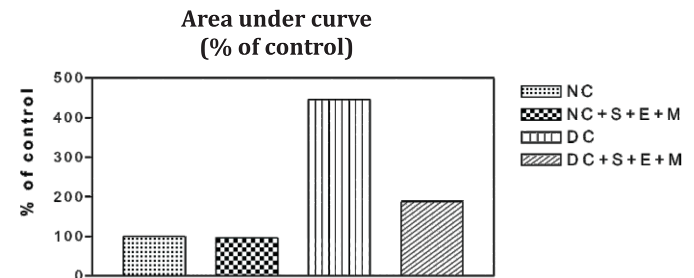
3.2.Glucose tolerance test (GTT) (Figures. 1,2 and Table 2)

Figure 1. Serum glucose levels in response to oral glucose tolerance test (OGTT) within a time range of 0 to 120 minutes in all the experimental groups.



Data are expressed as Mean±SE
NC = Non- diabetic control, NC+S+E+M = non - diabetic control+polyherbal extract+swimming+melatonin DC= diabetic control, DC+S+E+M = diabetic control+ swimming+polyherbal extract+ melatonin
,c) p<0.01 compared to NC and @) p< 0.01 compared to DC

Figure 2: Area under curve for OGTT in all experimental group



NC = Non diabetic control, NC+S+E+M = Non - diabetic control+polyherbal extract+swimming+melatonin DC= diabetic control, DC+S+E+M = diabetic control+ swimming+polyherbal extract+ melatonin

Table 2. Elevation and clearance rates (mg/min) of glucose during OGTT in exercised and extract and melatonin treated diabetic and non diabetic rats.

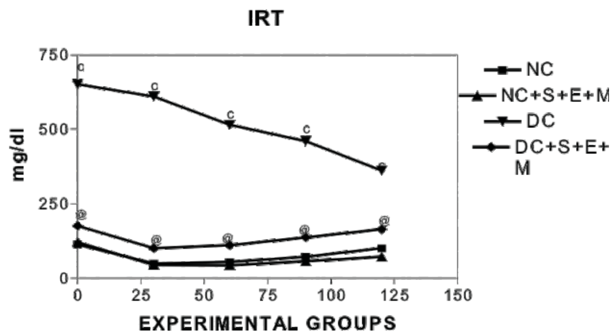
Oral glucose tolerance rate		
Groups	Rate of elevation	Rate of clearance
NC	1.21	0.50
NC+S+PE+M	1.43	0.85
DC	9.13	0.97
DC+ S+PE+M	11.46	3.65

NC = Non - diabetic Control, NC+S+E+M = Non - diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin

The glucose tolerance curves showed a poor diabetic curve, which was bettered closer to non-diabetic level by combinational therapy with S+PE+M. The area under the curve for DC+S+PE+M was almost as low as that of NC animals. The glucose elevation (per minute glucose elevation) to clearance (per minute glucose lowering) ratio (E:C) of DC+S+PE+M animals was as close as to that of non-diabetic animals, marked by a significantly high clearance rate.

3.3.Insulin response test (IRT) (Figures. 3, 4 and Table 3)

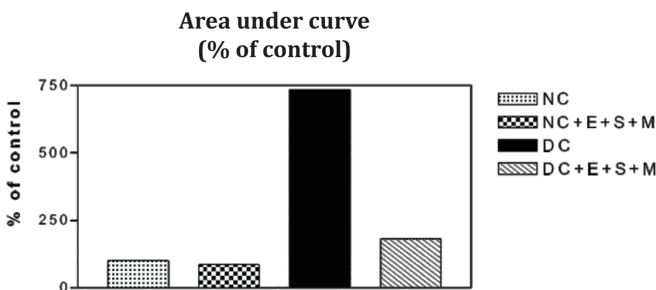
Figure 3: Serum glucose levels in response to insulin administration within a time range of 0 to 120 minutes of all the experimental groups



Data are expressed as Mean±SE
NC = Non - diabetic Control, NC+S+E+M = Non - diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin
c) $p<0.01$ compared to NC and @) $p< 0.01$ compared to DC

Figure 4: Area under curve for IRT in all experimental groups

Area under curve (% of control)



NC = Non Diabetic Control, NC+S+E+M = Non Diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin

Table 3. Clearance and elevation rates (mg/min) of glucose during IRT in exercised and extract and melatonin treated diabetic and non diabetic rats.

	Insulin response test	
Groups	Rate of elevation	Rate of clearance
NC	2.1	0.56
NC+S+PE+M	1.27	0.472
DC	2.4	0
DC+ S+PE+M	2.48	0.70

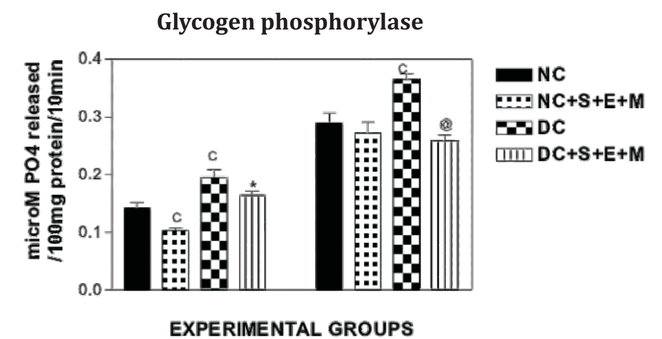
NC = Non - diabetic Control, NC+S+E+M = Non - diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin

Like the glucose tolerance curve, even the insulin response curve of DC+S+PE+S animals was noted to be close to and as similar to that of NC animals. The area under the curve was also significantly minimal and very much closer to the non-diabetic one. The glucose clearance to elevation rate (C: E) was also similar to NC and the insulin induced glucose clearance was maximal in these animals.

3.4.Carbohydrate metabolism (Figures. 5, 6 and Table 4)

Figure 5. Hepatic and muscle glycogen phosphorylase activity in exercised and extract and melatonin treated non diabetic and diabetic rats

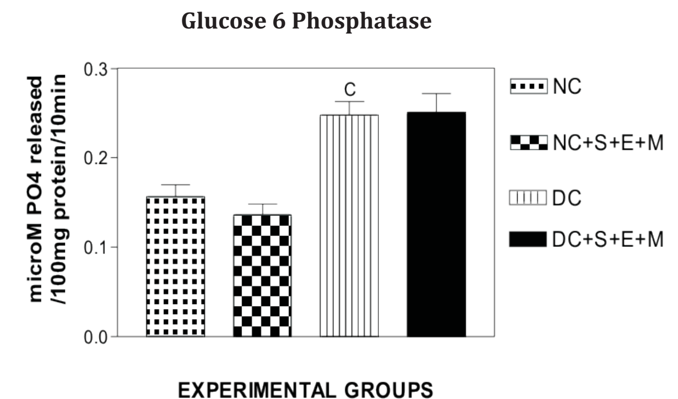
Glycogen phosphorylase



Data are expressed as Mean±SE
NC = Non - diabetic Control, NC+S+E+M = Non - diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin
c) $p<0.01$ compared to NC and *$p<0.05$, @) $p< 0.01$ compared to DC

Figure 6. Hepatic Glucose-6-phosphatase activity in exercised and extract and melatonin treated non diabetic and diabetic rats

Glucose 6 Phosphatase



Data are expressed as Mean±SE
NC = Non-Diabetic Control, NC+S+E+M = Non-Diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin
c) $p<0.01$ compared to NC

Table 4. Tissue protein and glycogen contents (mg/100 mg tissue) in control and treated diabetic and non diabetic rats

	Protein			Glycogen	
Groups	Liver	Muscle	Kidney	Liver	Muscle
NC	16.39±1.24	9.76±1.41	10.38±1.10	2.17±0.10	0.92±0.03
NC+S+PE+M	12.17±0.58^{c}	6.79±0.48^{a}	9.80±0.91	2.68±0.07^{c}	1.19±0.18
DC	13.90±0.87	5.50±0.7^{c}	10.04±1.58	1.79±0.06^{d}	0.58±0.05^{e}
DC+ S+PE+M	8.38±0.38$^{@}$	4.72±0.45	5.80±0.39$^{@}$	1.95±0.09*	0.83±0.02$^{@}$

Data are expressed as Mean±SE
NC = Non -diabetic Control, NC+S+E+M = Non -diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin
a) $p<0.05$ c) $p<0.01$, d) $p< 0.005$, e) $p< 0.0005$ compared to NC and * $p<0.05$, @) $p< 0.01$ compared to DC

Whereas the diabetic animals showed significantly lowered hepatic and muscle glycogen contents, combinational therapy with S+PE+M showed increment in both NC and DC animals. Reciprocal changes in phosphorylase activity in the form of increase in DC animals and decrease in NC+S+PE+M and DC+S+PE+M animals were the feature. Similar pattern of changes was recorded for G6Pase as well.

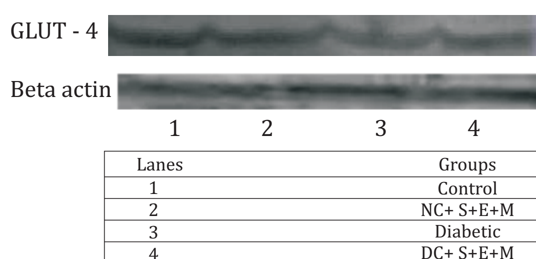
3.5.Tissue Protein content (Table 4)

Tissue protein contents of liver, muscle and kidney were significantly lower in DC animals and combination therapy of both NC and DC animals resulted in decrease of tissue protein contents.

3.6.Immunoblot analysis of cytosolic GLUT 4 (Figs, 7A, B)

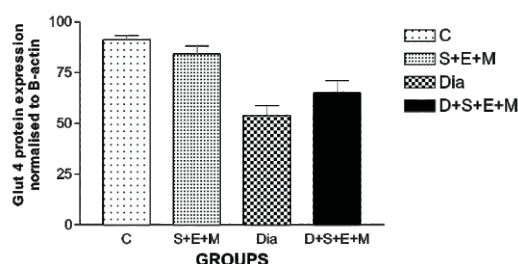
Fig. 7 (A) Immunoblot analysis of Glut-4 protein expression. (B) Semi quantification analysis of Glut-4 protein using scanning densitometry. Signals of Glut-4 in immunoblot were quantified arbitrarily. Bars represent means ± S.E. of independent experiments and a representative immunoblot is shown here.

Figure. 7A.



Lanes	Groups
1	Control
2	NC+ S+E+M
3	Diabetic
4	DC+ S+E+M

Figure. 7B.



Data are expressed as Mean±SE
NC = Non - diabetic Control, NC+S+E+M = Non - diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin c) $p<0.01$ compared to NC AND # $p<0.025$ compared to DC.

The immunoblot analysis of cytosolic GLUT 4 showed a significant decrement in diabetic animals, which was reversed effectively with S+PE+M combinational therapy.

4.Discussion

The present study on multi-dimensional therapeutic approach for combating experimentally induced Type I diabetes has revealed a much better glycaemic regulation. The excellent glucoregulatory effect of polyherbal therapy with melatonin supplementation and an exercise regimen (NC.S+M+PE and DC.S+M+PE), is best revealed by the normoglycaemic status of overnight starved experimental DC animals and a mere 86% hyperglycaemia in the fed experimental DC animals compared to 595% hyperglycaemia in DC animals. The average glycaemic status of fasted and fed states of DC.S+M+PE animals was only marginally higher than NC animals (139.66 v/s 103.66). Apparently, the S+M+PE schedule is very effective in managing diabetic hyperglycaemia even better than S+PE schedule employed previously [18] attesting to the complementing and additive role of melatonin over and above that of S+PE in diabetic glucoregulation. This becomes clear from the glucose tolerance and insulin response curves which are much closer to those of NC rather than DC. The glucose clearance rates of DC.S+M+PE animals under both glucose challenge and exogenous insulin, are much greater than both NC and DC animals, alluding to increased insulin release consequent to glucose challenge as well as, increased sensitivity to exogenous insulin in these animals. Moreover, the C/E ratio under GTT and E/C ratio under IRT of these animals are again much closer to NC suggesting near normal glucoregulation under the multidimensional therapy. It is also pertinent to note that, the C/E and E/C ratios of DC.S+M+PE animals are relatively much better than the ratios for all other combinations of DC.S+M, DC.S+PE and DC.M+PE [18] or even M and S alone [unpublished data]. The currently observed near normal glucoregulation under the multi-therapeutic combination appears to provide evidence for the additive anti-hyperglycaemic effects of plants like *Coccinia indica, Ocimum sactum, Annona squamosa, Murraya koenigii* and *Mangifera indica* [26 -35], exercise and melatonin [11, 36, 37, 33, 13,]. Apart from increased insulin sensitivity inferred earlier with reference to S, PE and S+PE and S+M [18,unpublished data] the presently observed increased insulin levels in both NC and DC animals subjected to S+M+PE clearly provides substantiation for the observed near normal glucoregulation and, is a novel effect of the combination therapy, not achieved by any of the other combinations. Apparently, the present multi-therapeutic combination seems to combine the cytoprotective effects of M [38], increased insulin sensitivity due to S and the rejuvenating

effect on β cells along with insulin secretion promoting activity of *Annona squamosa* and *Murraya koenigii* [39, 32]. A discordant note however, is the unchanged G6pase activity despite the recorded glucoregulation, which would tend to contradict the anti-hyperglycaemic effect of S+M+PE and indicate gluconeogenesis. The persistent gluconeogenic effect is well corroborated by the concurrent decrease in tissue protein content. Despite the persistent gluconeogenic effect, normoglycaemia seems to be achieved not only through the increased insulin production and sensitivity as inferred earlier, but also due to reduced intestinal absorption of glucose, a property of Mangifera indica [26, 27, 31], probably further exacerbated by as yet inexplicable interactive effect of the components of the multi-therapeutic regimen.

The increased insulin titre and sensitivity find reflection in the observed increased tissue glycogen content as well as decreased glycogen phosphorylase activity. Increased glycogenesis seems to be one of the mechanisms by which glucoregulation is achieved and, in the context of the persistent gluconeogeneis, even increased glucose oxidation can also be presumed as distinct possibility. Withdrawal of blood glucose for glycogenesis can be attributed to the glycogenesis promoting effects of *Murraya koenigii* and *Coccinia indica* [40, 41] and of melatonin [42]. The glycogenic potential of M is attributed to activation of PKC - Akt - GSK 3β dependent pathway through activation of M receptor [43]. Another important mechanism of glucose clearance involves increased peripheral glucose uptake by muscle and adipose tissue through activation of insulin receptor downstream signaling entities like insulin receptor substrate (IRS-1), PRPP kinase, PPAR γ, PI3 kinase and membrane GLUT- 4. Diabetes induced impaired GLUT-4 translocation and, attenuated expression of PI3 kinase and PPAR γ and, up regulation of GLUT-4 translocation and augmented expression of all the above signaling components by insulin, have been clearly documented [44, 45, 46, 47, 48, 49]. Presently observed increase in sarcoplasmic GLUT -4 expression in DC animals treated with S+M+PE is quite self-explanatory in the above context and, provides adequate glucose molecules for glycogenesis and oxidation. In support of the currently recorded increase in GLUT-4 expression and glucose uptake, are the reports of exercise induced increase in GLUT-4 expression [50, 51] and M induced IRS-1 phosphorylation [52]. It could be safely assumed from these that, S+M+PE exerts favourable influence on glucose uptake and utilization by way of increased insulin titre and sensitivity as well as up regulation of GLUT-4.

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