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Original Article

Supplementation with a polyherbal extract and melatonin together with exercise effectively corrects dyslipidemia but with some incompetence in reversing antioxidant status and hepatic and renal dysfunction

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ABSTRACT

The present study is a multi-dimensional approach aimed at assessing the efficacy of a polyherbal extract in combination with melatonin intake, as a safe natural anti-oxidant of the body, along with a regimen of swimming exercise. Adult albino rats were made diabetic by a single intraperitoneal injection of alloxan (120 mg / kg body weight). 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 lipid metabolism, enzymic and non enzymic markers of oxidative stress and serum markers of hepatic and renal dysfunction were evaluated. Results revealed a differential response in the form of lowered tissue lipid and cholesterol load though with certain degree of renal oxidative stress and abnormal renal function and a marginally higher serum levels of cholesterol and lipid. In contrast to non-diabetic animals, diabetic ones were more sensitive to S+M+PE treatment schedule as marked by the significant depletion in tissue lipid and cholesterol contents as well as anti hypertriglyceridemic and hypercholesterolemic effects. The present study involving a combination therapy seems to cause some oxidative stress as marked by significant decrement in CAT and SOD activities of liver, muscle and kidney along with GSH depletion with no increase in LPO. Increase in serum Corticosterone level could be speculated to be contributor of oxidative stress. In conclusion it can be said that S+M+PE is an effective combination therapy exerting antilipidemic effects but with some incompetence in containing sub adaptive swimming exercise induced oxidative stress and hepatic and renal dysfunctions. A properly worked out intensity and duration dependent adaptive exercise schedule is likely to overcome these shortcomings. Such an exercise schedule needs to be then studied in conjunction with melatonin supplementation and PE treatment.

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1. Introduction

In one of our earlier study, a cost effective polyherbal preparation aiming to combine the specific attributes of seven individual plants, all commonly available or accessible, had been evaluated with an added dimension of exercise, in alloxan induced Type I diabetic rats [1, 2] The objective of the entire exercise was to combat diabetic complications at multiple foci as no single drug in vogue is effective in tackling the defects in diabetes at all levels, besides being beset with serious side effects and long term inefficacy. As a sequel to it, the present study deals with a combination of polyherbal preparation, Swimming exercise and melatonin as an ideal mix in alloxanized Type I diabetic rats.

Melatonin (N-Acetyl 5-methoxytryptamine), is a ubiquitous molecule, which plays an important role in many physiological functions. The antioxidant properties of melatonin are a recently discovered fact [3]. Pineal is the main source of melatonin secretion and it regulates the circadian rhythm, with low serum levels during the day and higher levels at night. Melatonin, as well as its metabolites [4], possess redox properties because of the

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presence of an electron rich system which allows these molecules to act as electron donors. It has been reported that melatonin can effectively normalize the impaired antioxidative status in rats with streptozotocin-induced diabetes [5].

Healthy lifestyle is always a way of keeping off the onset of many metabolic disorders like diabetes and obesity, and many patients have been benefited by the inclusion of physical activities as part of therapy for these conditions, as per a study carried out by Yuzo Sato et al. [6]. Physical activities have definite role in preventing the onset of diabetes in individuals heading towards it, besides many other benefits too.

The present study is a multi-dimensional approach aimed at assessing the efficacy of a polyherbal extract with apparent differential effects,in combination with melatonin intake as a safe natural anti-oxidant of the body along with regimen of swimming exercise. Since all combination approaches or schedules are not likely to be an ideal mix, development of an ideal mix or multidimentional therapeutic approach aimed at the generation of a perfect blend of complementing therapy, is possible only by experimentally trying out different combinations. The present evaluation involving a polyherbal extract alongwith supplementation with melatonin and an exercise regimen is an attempt in this direction to gauge the scientific merit of such a combination therapy for Type I 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. Authentication of the plant material after collection was done 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 plucked and separated from the twig was used. The preparation of Polyherbal extract was as per to the method described in our earlier work [1, 2].

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. [1, 2].

2.4. Experimental animals

Female Wistar rats (200-250g) were housed in the departmental animal house under controlled room temperature (21 ± 2^0C). 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 induction was done using Alloxan according to the preocedure described in our earlier study [1, 2].Animals having fasting blood glucose levels higher than 300 mg/dl were only considered 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 treated with a combination of swimming exercise (S) for a period 15 days along with administration of polyherbal extract (PE) at a dose of 250mg/kg body weight at 8:00 hrs and melatonin (M) 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 treated with a combination of S for a period 15 days along with administration of PE at a dose of 250mg/kg body weight at 8:00 hrs and M at a dose of 1mg/kg body weight at 18:00hrs daily for a period of 15 days.

2.6. Biochemical analysis

At the end of a 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° for further analysis. Beneficial effect of PE over diabetes induced oxidative stress (ROS) was determined by assessing the level of LPO and by estimating the endogenous enzymatic and non-enzymatic antioxidant status. Lipid peroxidation (LPO) was determined as per the method described by Beuge and Aust [7], Reduced glutathione (GSH) by Beutler *et al.* [8], Superoxide Dismutase (SOD) by Marklund and Marklund [9], Catalase by Sinha [10] and Glutathione Peroxidase (GPx) by Rotruck et al. [11].

All biochemical parameters and hormones were assayed using relevant kits as mentioned below:

a) Insulin (MERCODIA, Sweden). b) Cort and P4(Immuno-Technology & Steroid Laboratory Department of Reproductive Biomedicine, National Institute of Health and Family Welfare. Munirka, New Delhi). c) E2 (Biocheck Inc, California). d) Serum cholesterol (Accurex biomedical Pvt Ltd.). e) Serum Triglyceride (Accurex biomedical Pvt Ltd.). f) HDL (Nicolas Piramal India Ltd.). g) SGPT (Agappe Diagnostics Ltd.). h) SGOT (Crest Biosystem Ltd.). i) Alkaline Phosphatase (ALP) (Rekon diagnostics Pvt Ltd.). j) Acid Phosphatase (ACP) (Aspen Laboratories.). Tissue cholesterol and lipids were assayed by the methods of Crawford [12] and Folch *et al.* [13] respectively.

2.7. 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.Tissue lipid and cholesterol contents (Table 1)

Both cholesterol and lipid contents of liver, muscle and kidney cholesterol and lipid contents were increased significantly in diabetic animals. Whereas combinational therapy with S+PE+M significantly decreased tissue lipid contents in both NC and DC animals, the same decreased tissue cholesterol contents only in DC animals while, increasing the contents in NC animals.

Table 1. Tissue Cholesterol and Lipid contents (mg/100mg tissue) in exercised and extract and melatonin treated diabetic and non diabetic rats

GROUPS	CHOLESTEROL			LIPID		
	Liver	Muscle	Kidney	Liver	Muscle	Kidney
NC	0.28±0.005	0.12±0.01	0.37±0.03	4.21±0.72	1.56±0.43	0.73±0.06
NC+S+PE+M	0.44±0.03	0.19±0.044	0.45±0.02[a]	3.81±0.44[b]	1.10±0.38	0.65±0.06
DC	0.60±0.004[e]	0.29±0.003[e]	0.58±0.004[e]	6.32±0.02[e]	2.08±0.41	0.93±0.04[e]
DC+S+PE+M	0.41±0.023	0.197±0.005[@]	0.507±0.01[@]	5.31±0.71[@]	1.17±0.50[@]	0.80±0.05

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, d) p< 0.005, e) p< 0.0005 compared to NC and @) p< 0.01compared to DC

3.2.Serum lipid profile (Table 2)

While the serum TG and Cholesterol fractions were significantly high in DC animals, combinational therapy decreased both significantly to near NC levels. Combinational therapy of NC animals increased serum TG level without altering serum cholesterol fractions.

Table 2. Serum lipid profile (mg/dl) of exercised and extract and melatonin treated non diabetic and diabetic rats

GROUPS	Cholesterol	Triglyceride	HDL	LDL	VLDL
NC	80±2.31	68.67±3.44	15±1.73	13.11±1.73	50.66±1.76
NC+S+PE+M	79±1.15	104.5±5.03[c]	48.34±2.24[c]	16.50±1.97	14.52±0.351[c]
DC	97.33±4.33[d]	140.66±2.33[e]	30.66±0.86[e]	22.22±2.89e	45.44±2.60
DC+S+PE+M	88.25±2.15*	85.47±1.08[@]	48.71±2.37[@]	20.49±0.92[@]	19.42±0.35[@]

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
d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05@) p< 0.01compared to DC

3.3. Serum hormonal profile (Table 3)

Insulin titre was significantly low in DC animals and, combinational therapy of both NC and DC animals significantly elevated serum insulin level. Corticosterone was significantly increased in both - NC +S+PE+M and DC animals, but with combination therapy of DC animals, Cort level was significantly decreased. Oestrogen was increased in all the experimental groups in the order DC+S+PE+M>DC>NC+S+PE+M. Progesterone showed a similar range of lowered level in all the experimental groups.

Table 3. Serum hormone profile of exercised and extract and melatonin treated non diabetic and diabetic rats

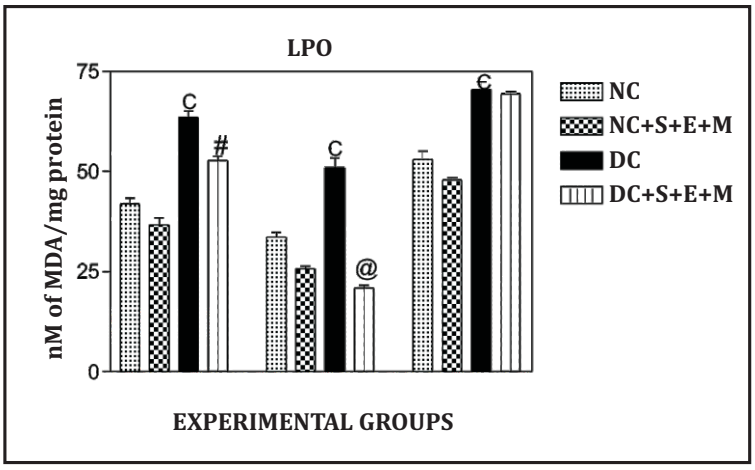
GROUPS	Corticosterone (ng/ml)	Estradiol (pg/ml)	Progesterone (ng/ml)
NC	8.38±0.59	0.19±0.008	66.68±3.48
NC+S+PE+M	18.54±0.05[c]	1.56±0.01[c]	50.857±0.09[c]
DC	24.66±1.45[e]	1.99±0.07[e]	54.22±1.74[d]
DC+S+PE+M	14.47±0.11[@]	2.17±0.037*	59.74±0.13[@]

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, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, @) p< 0.01compared to DC

3.4. Oxidative stress parameters

3.4.1. Lipid peroxidation (LPO): Liver, muscle and kidney (Fig. 1)

All three organs registered significantly elevated LPO levels and, combinational therapy lowered LPO levels significantly in both NC and DC animals, more prominently in liver and muscle.



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, @) p< 0.01compared to DC

3.5. Antioxidants

3.5.1. Non enzymatic antioxidants (Table 4); Reduced glutathione (GSH).

The most prominent change with reference to GSH was a significant decrease in all the three organs of diabetic animals and a significant increment in these animals when put on combinational therapy. There was no significant change in NC animals on combination therapy except for a tendency for increase in muscle and kidney and decrease in liver.

Table 4 Tissue non-enzymatic anti-oxidant status (g of GSH /min/mg protein) in exercised and extract and melatonin treated non diabetic and diabetic rats

	GSH		
	Liver	**Muscle**	**Kidney**
NC	31.14±2.58	14.58±1.51	25.03±1.15
NC+S+PE+M	26.80±1.40	15.21±0.92	28.42±0.69[b]
DC	11.00±1.28[e]	13.05±1.37	13.03±1.85[e]
DC+S+PE+M	17.09±0.85[@]	21.8±0.73[@]	18.08±0.90[#]

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<0.025e) p< 0.0005 compared to NC and,#p<0.025, @) p< 0.01comparedtoDC

3.5.2. Enzymatic antioxidants (Table 5): Catalase (Cat), superoxide dismutase (SOD) and Glutathione peroxidase (GPx)

A common change noticeable was the significantly decreased activity of all the three enzymes in all the three organs of diabetic animals. Diabetic animals subjected to combination therapy showed increased activity of GPx in all the organs, of Cat in liver and muscle with decrease in kidney and of SOD, which remained unchanged. In contrast, combinational therapy of NC animals resulted in significant decrease in Cat and SOD activities and increase in GPx activity.

Table 5. Enzymatic anti-oxidant status of exercised and extract and melatonin treated non-diabetic and diabetic rats

	SOD U/mg protein			CATALASE (mM of H_2O_2 decomposed/mg protein/min),			GPx (g of GSH/min/mg protein)		
GROUPS	**Liver**	**Muscle**	**Kidney**	**Liver**	**Muscle**	**Kidney**	**Liver**	**Muscle**	**Kidney**
NC	8.17±0.59	10.36±0.60	5.38±0.39	53.95±2.54	73.11±2.59	26.74±3.10	4.59±0.83	12.45±1.60	2.16±0.18
NC+S+PE+M	6.17±0.20[c]	8.54±0.23c	3.49±0.21c	42.61±0.57c	63.7±2.09c	17.42±0.85c	6.45±0.33	16.63±0.39b	8.37±0.37c
DC	4.63±0.44[e]	6.55±0.47e	2.7±0.14e	22.96±2.08e	49.83±2.34e	13.40±0.87e	2.68±0.30b	7.47±0.89c	1.17±0.24
DC+S+PE+M	4.65±0.24	4.17±0.03@	2.38±0.24	24.13±0.83	61.75±1.07@	8.65±0.30@	4.24±0.28	16.86±0.66@	9.86±0.40@

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, b)p<0.025,c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05,#p<0.025, @) p< 0.01, p<0.005) p< 0.0005 compared to DC

3.6. Serum markers of hepatic function (Table 6): SGPT, SGOT, ALP and ACP

Some specific effects of S+PE+M in hepatic function of NC animals seem indicated by the significant decrease in SGPT and ALP but marginal increase of SGOT and ACP. Diabetic animals showed a uniform effect of significant increment of all hepatic markers and significant reduction on subjecting these animals to combinational therapy.

Table 6. Serum Markers (U/L) of Hepatic Dysfunction in exercised and extract and melatonin diabetic and non diabetic rats

Groups	SGPT	SGOT	ALP	ACP
NC	40±4.04	71.33±2.96	204±2.64	8.5±0.86
NC+S+PE+M	21.01±0.56[c]	82.14±0.58[c]	67.28±0.92[c]	10.37±0.85
DC	125±5.86[e]	290.66±5.79[e]	471.66±2.33[e]	12.2±0.61[d]
DC+ S+PE+M	55.14±0.49[@]	221.66±2.02[@]	387.66±3.18[@]	10.23±0.43[#]

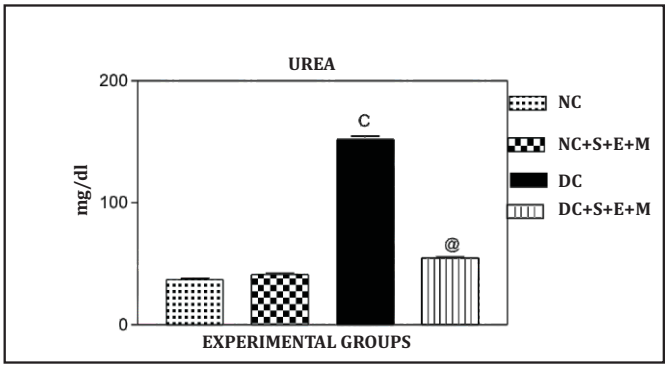
Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+E+M = Non Diabetic Control+Polyherbal Extract+Swimming+Melatonin DC= Diabetic Control, DC+S+E+M = Diabetic Control+ Swimming+Polyherbal Extract+ Melatonin
c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and,#p<0.025, @) p< 0.01compared to DC

3.7. Serum markers of renal function (Figure 2 and 3)

3.7.1 Urea and Creatinine

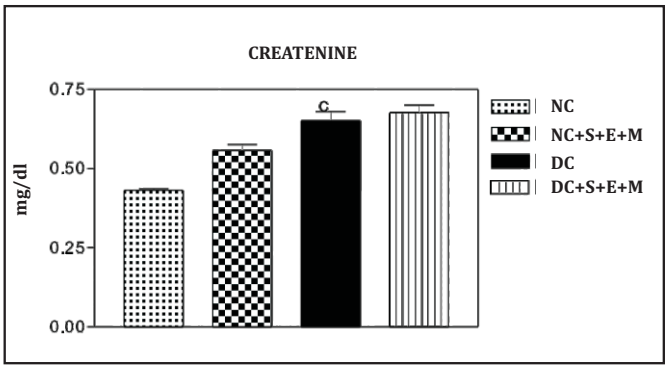
Diabetic animals were marked by significant increment in the serum markers of renal function. Whereas urea showed significant reduction on subjecting these animals to combinational therapy, creatinine level remained unchanged. Non-diabetic animals subjected to combinational therapy also showed a tendency for marginal increase in both urea and creatinine.

Figure 2. Serum Urea level in exercised and extract and melatonin treated non diabetic and diabetic rats



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<0.01compared to NC and @) p< 0.01compared to DC

Figure 3. Serum Creatinine level in exercised and extract and melatonin treated non diabetic and diabetic rats



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

4. Discussion

The present study on multi-dimensional therapeutic approach for combating experimentally induced Type I diabetes, has revealed a differential response in the form of lowered tissue lipid and cholesterol load, though with certain degree of renal oxidative stress and abnormal renal function and, marginally higher serum levels of cholesterol and lipid.

Dyslipidemia is a diabetic manifestation and, the same is an observed consequence of alloxan induced Type I diabetes in the present study, as marked by elevated serum TG, TC, LDL and VLDL levels along with increased tissue cholesterol contents. The presently observed increase in serum TG coupled with significant depletion of tissue lipids in NC.S+M+PE animals is suggestive of increased lipid mobilization, an interactive effect of S+M as seen previously [unpublished data] and, a consequence of exercise induced increase in energy demand. However, under utilization of mobilized lipids can be presumed from the recorded depletion in tissue lipids coupled with increased serum TG level. Lipolysis seems to far outweigh lipid utilization, probably a non-adaptive effect of exercise and, which is likely to stabilize with prolonged exercise, an adaptation for lipid utilization. Apparently, metabolic adaptation with prolonged exercise, is expected to lower serum TG level, as has been shown by other workers studying exercise adaptation [14]. A possible explanation for the observed higher serum TG level could be a higher level of glucagon and catecholamines in response to sub-maximal non-adaptive exercise schedule. The observation of Winder et al. [15] of increased plasma glucagon and catecholamines in individuals subjected to sub maximal exercise provides support to this contention. The currently observed changes in serum TG and tissue lipids are more of a potentiated S effect in presence of M [unpublished data]. In contrast, the increased tissue cholesterol contents and decreased serum cholesterol level are more of M effect slightly toned down by S [unpublished data]. In DC animals, S+M+PE seems to have greater tissue lipid and cholesterol lowering effects from the high diabetic levels compared to S+M treatment. Even serum TG and cholesterol levels of DC animals are lowered more by S+M+PE compared to S+M treatment. In contrast to non-diabetic animals, diabetic ones seem to be more sensitive to S+M+PE reatment schedule as marked by the significant depletion in tissue lipid and cholesterol contents as well as anti hypertriglyceridimic and anti-hypercholesterolemic effects. The presence of PE seems to complement S+M and alleviate diabetic hyperlipidemia and hypercholesterolemia, more than that seen for S+M treatment [unpublished data]. The lipid and cholesterol lowering effects of *Murraya koenigii* [16, 17], *Ocimum sanctum* [18], *Cassia fistula* [19] and *Annona squamosa* [20] appear pertinent in this context and, probably the effect of these plants is potentiated by simultaneous supplementation with M, and an exercise regimen.

Growing evidence indicates enhanced oxidative stress in diabetic manifestations [21,22] and as such, the involvement of free radicals in type I diabetic animal models has been shown [23,22]. Alloxan or streptozotocin induced type I animal models have shown decreased total SOD activity in almost all organs except for brain and lung [24]. Diabetic patients present elevated lipid peroxidation level [25,26] and, this has been related with the hyperglycaemic status [27]. The present study involving a combination therapy seems to cause some oxidative stress as marked by significant decrement in Cat and SOD activities of liver, muscle and kidney along with GSH depletion. Though these changes tend to suggest oxidative stress, no increase in LPO and even a decrease in its levels, are perplexing and suggest the efficient participation of endogenous antioxidants in neutralizing any irritant pro-oxidants that may purportedly be formed by sub maximal swimming exercise and the combination of herbal principles [1, 2].Increase in serum corticosterone level also probably contributes to the oxidative stress Apparently, NC animals are exposed to certain degree of oxidative stress which is effectively countermanded by the actions of SOD and Cat and by using the reductive power of GSH. Significantly, increased GPx activity in all the three organs suggests an adaptive mechanism in operation to regenerate GSH. Studies on exercise induced alterations in endogenous antioxidant status and oxidative stress are inconclusive, with few studies suggesting generation of some oxidative stress and others suggesting upregulation of antioxidant status [28].Presumably, initial phases of submaximal training exercise prior to adaptation may result in some oxidative stress while, long term exercise may lead to adaptation and favorable whole body antioxidant status. In contrast to NC animals, S+M+PE treatment schedule seems to have a much favorable effect in DC animals. Significant decrement in LPO with increment in GSH and GPx suggests amelioration of diabetes induced oxidative stress. Persistence of SOD and Cat inactivation almost to the same extent as in DC animals indicate inability of the treatment schedule to completely restore the endogenous antioxidant status. The overall endogenous scenario of DC.S+M+PE animals seems to be relatively better than that of DC.S+M animals and, maximal oxidative stress seems to be affecting liver and kidney rather than muscle. The favorable influence of the treatment schedule to counter diabetes induced oxidative stress may be accredited to exercise induced amelioration[29, 22, 30] and the antioxidant effects of *Coccinia indica* [31], *ocimum sanctum* [32], *Cassia fistula* [33, 34], *Mangifera indica* [35] and *Annona squamosa* [36]. In this context, favorable influence of S+M+PE could be considered as a cumulative effect of the antioxidant ability of S,M and PE. Even the decreased serum corticosterone level and highly elevated E2 titre could also be implicated in the alleviation of diabetic oxidative stress.

In keeping with the observed oxidative stress even in NC animals, hepatic and renal functions also seem to be affected as marked by increased serum levels of urea and creatinine (Kidney) and SGOT and ACP (Liver). Since similar effects were also found in NC.S+M animals, it points towards S as the culprit as, significant increment in these markers was recorded in NC.S animals (Singh, 2010). It is likely that, the intensity and duration of exercise employed herein may be sub-adaptive, imposing some strain on hepatic and renal functions. Interestingly, both NC.S+M [unpublished data] and NC.S+M+PE (present study) have shown significantly lesser elevation in serum markers of hepatic and renal functions compared to NC.S animals [unpublished data]. This suggests that, M and PE can alleviate to a greater extent the hepatic and renal stress imposed by sub adaptive swimming exercise. As against this, S+M+PE seems to be very effective in overcoming diabetes induced hepatic and renal dysfunctioning. In fact, M and PE together are more effective than M alone in reversing the serum markers of hepatic and renal dysfunctioning. The remarkable improvement in hepatic and renal functions in DC.S+M+PE animals can be accredited to the alleviating influence of *Annona squamosa* [37], *Murraya konigii* [16, 17], *Ocimum scactum* [32], *Mangifera indica* [35] and *Cassia fistula* [38] as well as of the cytoprotective effects of melatonin [22, 39, 40, 41]. The concurrently observed decrement in serum corticosterone titre and increment in estrogen level are supportive hormonal *milieu* with favorable disposition for amelioration of oxidative stress and hepatic and renal dysfunctions.

5. Conclusion

In conclusion, it can be said that the combination therapy involving polyherbal extract, melatonin and swimming exercise, has significant potential to combat diabetic dyslipidemia, tissue oxidative stress and hepatic and renal dysfunctions, though with a note that the exercise regimen need to be adaptive and not sub adaptive essentially in relation to oxidative stress and renal functions.

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