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Original Article

EVALUATION ON THE EFFICACY OF A POLY HERBAL SUPPLEMENT ALONG WITH EXERCISE IN ALLEVIATING DYSLIPIDEMIA, OXIDATIVE STRESS AND HEPATIC AND RENAL TOXICITY ASSOCIATED WITH TYPE -1 DIABETES

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ABSTRACT: ***Modification of dietary habits and life style changes such as, regular exercise together with mixed formulations containing blood sugar lowering herbs in combination with antioxidants and detoxicants, have been incorporated in the management of type -1 diabetes. Aqueous extract of 7 plants (Annona squamosa(Annonaceae), Cassia fistula, Coccinia indica, Mangifera indica, Ocimum sanctum, Lagerstroemia losflos-reginee, and Murraya koenigii) (PE) together with swimming exercise (S) has been employed in the present study to evaluate the efficacy against dyslipidemia and oxidative stress associated with type-1 diabetes. To this effect, adult albino rats were made diabetic (DC) by a single i.p injection of alloxan (120 mg / kg body weight). Animals having blood glucose level of 300 mg / dl or higher were considered diabetic. Control and experimental animals were supplemented with PE and subjected to swimming exercise of 30 minutes duration for 15 days. Animals were sacrificed on the 16th day and, various parameters related to oxidative stress (SOD, CAT, GPx, GSH and LPO) and lipid metabolism (Total lipids, TG, TC, LDL, VDL, and VLDL) were evaluated. Diabetic animals showed down regulated antioxidant defence and dysregulation of lipid metabolism. Both PE and S+PE restored the level of antioxidants and markers of lipid metabolism to near normal levels. Overall, the present study provides support and evidence for consideration of a therapeutic approach combining the beneficial effects of a polyherbal preparation in association with adaptive physical activity for alleviating symptoms of tissue oxidative damage and disorders of lipid metabolism.***

Keywords: Diabetes, Herbal extract, Alloxan, Exercise.

INTRODUCTION

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 a part of their therapy for the mentioned disorders, as per a study carried by Sato et al. [1]. Indian system of medicine is one of the oldest and, there are more than 100 medicinal plants mentioned in this system including folklore medicines for treatment of diabetic complications; theses mentioned plants are active at their best either individually or in combination [2]. India has a very long and safe history of usage of many herbal drugs as, we have officially recognized system of health care like Ayurveda, Yoga, Unani, Siddha, Homeopathy and, Naturopathy, and more than 500,000 non allopathic practitioners are trained in more than 400 medical colleges across the country. Hence, this system of therapy cannot rightly be considered as traditional or folklore herbal practices [3]. Many active ingredients of herbal formulations have

antioxidant property. Oxidative stress is the causative agent behind development and progression of several diseases including diabetes. Hence, any treatment regime for diabetes should essentially include measures to check the surge in free radicals thereby preventing tissue oxidative damage. The present study involves a polyherbal aqueous extract (PE), using leaves and seeds of different plants like *Annona squamosa(Annonaceae), Cassia fistula, Coccinia indica, Mangifera indica, Ocimum sanctum, Lagerstroemia losflos-reginee*, and *Murraya koenigii*. The plants used in the present study have blood glucose lowering effect in diabetic animals and, some of them also have anti-lipedemic and cholesterol lowering effects. Biochemical evaluation of antidiabetogenic properties of some of these plants have been carried out in streptozotocin-induced diabetic rats [4]. A major objective of selecting these plants is that they are commonly available with minimal or no cost of procurance and hence very cost effective. Apart form primary consequences like insulin insufficiency and hyperglycemia, secondary consequences like metabolic derangement, oxidative stress, hyperlipidemia etc. contribute to many other manifestations affecting the cardiovascular system, kidney, retina, lens and skin. Overall, quality of life suffers and longevity gets curtailed [5]. Aurvedic remedies for diabetes are usually mixed formulations containing blood sugar lowering herbs in combination with immunomodulators, diuretics and detoxicants.The rationale behind such formulations is provided by modern research, which documents that, immune process plays a predominant role in the destruction of beta cells and features predominantly in the progression of the disease and its secondary complications [6]. The present study was designed with the objective of elucidating the therapeutic efficacy of the polyherbal extract (PE) in combination with swimming exercise (S) in rats rendered diabetic by evaluating the dysregulation in lipid metabolism, degree of oxidative stress and hepatic and renal toxicity parameters. Swimming exercise was incorporated along with PE supplementation as, exercise has been known to have beneficial effects in diabetes management and in fact, swimming exercise has been recorded to have favorable effects in alloxan induced diabetic rats in terms of diabetic manifestations [7].

MATERIALS AND METHODS

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).

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 soxhelt at boiling temperature (100º C) 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 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.

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.

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.

Induction of Type I diabetes: Diabetes was induced in experimental rats by single intraperitonial injection of alloxan (120 mg/kg body weight) dissolved in 0.1 M Citrate buffer, pH 4.5. Diabetes was confirmed in animals after seven days of alloxan administration, and blood samples were collected form retro-orbital sinus and analyzed for glucose levels. Animals having fasting blood glucose levels higher than 300 mg/dl were considered for experiments.

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^{o} for further analysis. Beneficial effect of PE over diabetes led reactive oxygen species (ROS) was determined by estimating enzymatic and non-enzymatic antioxidant status. Lipid peroxidation (LPO) was determined as per the method described by Beuge and Aust [8]. Reduced glutathione (GSH) by Beutler et al. [9], Superoxide Dismutase (SOD) by Marklund and Marklund [10], Catalase by Sinha [11], Glutathione Peroxidase (GPx) by Rotruck et al. [12].

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

a) Insulin (MERCODIA, Sweden). b) Corticosterone and Progesterone (Immuno-Technology & Steroid Laboratory Department of Reproductive Biomedicine, National Institute of Health and Family Welfare. Munirka, New Delhi). c) Estradiol (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 [13] and Folch et al.[14] respectively.

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.

RESULTS

Tissue Lipid and Cholesterol (Table 1)

Diabetic animals showed significant increment in hepatic, muscle and kidney cholesterol and lipid contents. Whereas PE decreased tissue cholesterol contents and PE+S increased the same in NC animals, both PE and PE+S decreased tissue cholesterol contents in DC animals. In contrast PE and PE+S decreased tissue lipid contents in increasing order in both NC and DC animals.

Serum Lipids (Table 2)

Both PE and PE+S decreased serum TG levels significantly with PE+S>PE in NC and DC animals. Serum TC, LDL and VLDL were increased in NC+PE and decreased in NC+PE+S. In contrast, both PE and PE+S decreased serum TC, LDL and VLDL in DC animals with the decrement in PE+S being relatively more than PE.

Serum Hormone Titres (Table 3)

Corticosterone (Cort.), Oesterogen (E_2), Progesterone (P_4) and Insulin.

Diabetic animals recorded significant increment in Cort. and E_2 and decrement in insulin and P_4. Whereas insulin titre decreased significantly in NC+PE and NC+S+PE animals, it showed gradual but significant increment in the order PE>PE+S in DC animals. Corticosterone showed a reversed set of changes in the form of increase in NC+PE and decrease in NC+S+PE and, decrease in DC+PE and increase in DC+S+PE. Whereas E_2 showed consistent increment from PE to PE+S in both NC and DC animals, P_4 showed consistent decrement from PE to PE+S in both NC and DC animals.

Oxidative stress parameters

Lipid peroxidation (LPO) (Figure 1):Though LPO was significantly increased in liver, muscle and kidney in DC animals, it was decreased consistently on PE and PE+S (in that order) in both NC and DC animals.

Non-Enzymatic anioxidants

Reduced Glutathione (GSH) (Table 4) : Though diabetic animals show a generalised significant decrease in GSH of liver, muscle and kidney, NC and DC animals showed gradual significant increment when supplemented with PE or also subjected to S

Table 1: Tissue Cholesterol and Lipid contents (mg/100mg tissue) in Control and Treated Rats

	CHOLESTEROL			LIPID		
Groups	**Liver**	**Muscle**	**Kidney**	**Liver**	**Muscle**	**Kidney**
NC	0.28±0.0052	0.124±0.010	0.37±0.030	4.21±0.71	1.56±0.43	0.72±0.06
NC+PE	0.14±0.00033 [c]	0.15±0.00034 [c]	0.19±0.0012 [c]	3.85±0.73	1.41±0.40	0.69±0.06
NC+S+PE	0.32±0.0058 [c]	0.22±0.00088 [c]	0.55±0.0057 [c]	3.58±0.067	1.39±0.29	0.63±0.05
DC	0.604±0.0044	0.293±0.0034	0.58±0.0046	6.32±0.81	2.08±0.31	0.93±0.08
DC+PE	0.456±0.00057 [@]	0.30±0.0062	0.48±0.026 [@]	5.81±0.68 [@]	1.87±0.21	0.89±0.07
DC+S+PE	0.435±0.0024 [@]	0.123±0.001156 [@]	0.37±0.00578 [@]	4.85±0.61 [@]	1.69±0.31	0.81±0.06

Data are expressed as Mean±SE

NC = Non Diabetic Control, NC+PE = Non Diabetic Control+Polyherbal Extract, NC+S+PE = Non Diabetic Control+Swimming+Polyherbal Extract, DC= Diabetic Control, DC+PE = Diabetic Control + Polyherbal Extarct and DC+S+E = Diabetic Control+ Swimming +Polyherbal Extract

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

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

Groups	**CHO**	**TG**	**LDL**	**VLDL**	**HDL**
NC	80±2.31	68.67±3.45	15±1.73	13.11±1.74	50.67±1.77
NC+PE	106.33±0.33 [c]	86.15±0.094 [c]	29.82±0.25	22.74±0.13 [c]	53.33±0.069 [c]
NC+S+PE	79.56±0.29	85.12±0.061 [c]	14.83±0.095	14±0.58 [c]	51.22±0.061
DC	97.33±4.34	140.67±2.34	30.66±0.87	22.22±2.90	45.33±2.60
DC+PE	87.89±0.59*	87.63±0.043 [@]	20.59±0.26	19.33±0.33 [@]	48.49±0.17 [@]
DC+S+PE	80.67±0.33 [@]	82.52±0.31 [@]	17.57±0.148	14.79±0.24 [@]	48.33±0.0088 [@]

Data are expressed as Mean±SE

NC = Non Diabetic Control, NC+PE = Non Diabetic Control+Polyherbal Extract, NC+S+PE = Non Diabetic Control+Swimming+Polyherbal Extract, DC= Diabetic Control, DC+PE = Diabetic Control + Polyherbal Extarct and DC+S+E = Diabetic Control+ Swimming +Polyherbal Extract

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

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

GROUPS	**INSULIN**	**CORTICOSTERONE**	**ESTRADIOL**	**PROGESTERONE**
NC	0.349±0.013	8.38±0.59	0.19±0.008	66.68±3.48
NC+PE	0.25±0.00578 [c]	10.61±0.037 [c]	0.21±0.00883	71.79±0.16
NC+S+PE	0.245±0.006 [c]	7.36±0.090	0.241±0.095	51.81±0.12 [c]
DC	0.164±0.013	24.66±1.45	1.99±0.07	54.22±1.74
DC+PE	0.170±0.000334	5.55±0.054 [@]	2.103±0.012	43.75±0.19 [@]
DC+S+PE	0.185±0.000334	14.64±0.070 [@]	3.11±0.052 [@]	36.58±0.05 [@]

Data are expressed as Mean±SE

NC = Non Diabetic Control, NC+PE = Non Diabetic Control+Polyherbal Extract, NC+S+PE = Non Diabetic Control+Swimming+Polyherbal Extract, DC= Diabetic Control, DC+PE = Diabetic Control + Polyherbal Extarct and DC+S+E = Diabetic Control+ Swimming +Polyherbal Extract

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

Table 4: Tissue non-enzymatic anti-oxidant status (mg/100 mg tissue) in exercised and extract treated non diabetic and diabetic rats

	GSH		
Groups	**Liver**	**Muscle**	**Kidney**
NC	31.15±2.58	14.58±1.51	25.03±1.15
NC+PE	35.24±0.11	14.8±0.16	31.13±0.55 [c]
NC+S+PE	35.18±0.19	14.49±0.14	27.89±0.17 [c]
DC	11.00±1.29	13.05±1.38	13.04±1.86
DC+PE	12.55±0.30	16.54±0.26[#]	17.30±0.079[@]
DC+S+PE	16.02±0.049[@]	18.61±0.26[@]	20.84±0.13[@]

Data are expressed as Mean±SE ; NC = Non Diabetic Control, NC+PE = Non Diabetic Control+Polyherbal Extract, NC+S+PE = Non Diabetic Control+Swimming+Polyherbal Extract, DC= Diabetic Control, DC+PE = Diabetic Control + Polyherbal Extarct and DC+S+E = Diabetic Control+ Swimming +Polyherbal Extract ; 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

Table 5: Tissue enzymatic anti-oxidant status of exercised and extract treated non-diabetic and diabetic treated rats

GROUPS	**SOD**			**CATALASE**			**GPx**		
	Liver	**Muscle**	**Kidney**	**Liver**	**Muscle**	**Kidney**	**Liver**	**Muscle**	**Kidney**
NC	8.17± 0.60	10.37± 0.60	5.38± 0.39	53.96± 2.54	73.12± 2.59	26.74± 1.94	4.59± 0.84	12.45± 1.61	2.16± 0.19
NC+PE	7.85± 0.015	11.47± 0.015[a]	5.48± 0.015	47.10± 0.066[b]	66.67± 0.28[b]	26.79± 0.22	9.91± 0.043 [c]	34.39± 0.48 [c]	8.45± 0.36 [c]
NC+S+PE	7.61± 0.012	11.88± 0.015[b]	6.02± 0.038	50.52± 0.30	68.73± 0.22	25.08± 0.20	11.97± 0.31 [c]	35.53± 0.41 [c]	10.5± 0.46 [c]
DC	4.64± 0.45	6.56± 0.48	2.72± 0.15	22.96± 2.09	49.83± 2.34	13.41± 0.87	2.68± 0.30	7.47± 0.89	1.18± 0.24
DC+PE	5.13± 0.0088	7.35± 0.029	3.05± 0.043*	25.61± 1.063	57.67± 0.22[@]	20.65± 0.25[@]	6.26± 0.11[@]	17.35± 0.15[@]	4.90± 0.13[@]
DC+S+PE	5.616± 0.071*	8.09± 0.0057[@]	3.92± 0.10[@]	29.78± 0.17[@]	68.49± 0.21[@]	20.60± 0.37[@]	7.37± 0.049[@]	19.91± 0.11[@]	7.9± 0.13[@]

Data are expressed as Mean±SE; NC = Non Diabetic Control, NC+PE = Non Diabetic Control+Polyherbal Extract, NC+S+PE = Non Diabetic Control+Swimming+Polyherbal Extract, DC= Diabetic Control, DC+PE = Diabetic Control + Polyherbal Extarct and DC+S+E = Diabetic Control+ Swimming +Polyherbal Extract ; 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

Table 6: Serum Markers of Hepatic Dysfunction in Control and Treated Rats

Groups	**SGPT**	**SGOT**	**ALP**	**ACP**
NC	40±4.05	71.33±2.97	204±2.65	8.5±0.87
NC+PE	38.33±0.33	222±0.58 [c]	121±0.58 [c]	10.56±0.033[b]
NC+S+PE	29.33±0.33[b]	157.66±0.33 [c]	108.33±0.33 [c]	9.6±0.058
DC	125±5.86	290.66±5.78	471.6667±2.34	12.2±0.61
DC+PE	42.33±0.33[@]	245.67±0.33[@]	207±0.58[@]	8.76±0.033[@]
DC+S+PE	33.33±0.33[@]	153.33±0.33[@]	139.33±0.33[@]	3.36±0.033[@]

Data are expressed as Mean±SE ; NC = Non Diabetic Control, NC+PE = Non Diabetic Control+Polyherbal Extract, NC+S+PE = Non Diabetic Control+Swimming+Polyherbal Extract, DC= Diabetic Control, DC+PE = Diabetic Control + Polyherbal Extarct and DC+S+E = Diabetic Control+ Swimming +Polyherbal Extract; 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

more prominently in DC animals as, the increment in NC+PE and NC+S+PE tended to remain the same.

Enzymatic Antioxidant status (Table 5) : Catalase (Cat), Superoxide Dismutase (SOD) and Glutathion Peroxidase (GPx). Diabetic animals showed significant decrement in the activity of all the three enzymes in all the three organs. Both Cat and GPx showed a generalised pattern of increasing activity with PE+S>PE in both NC and DC animals. However, SOD showed this trend only in DC animals as all the NC groups of animals tended to have same range of enzyme activity.

Serum Markers of Hepatic function

SGPT, SGOT, ALP and ACP (Table 6): Diabetic animals showed significantly increased serum levels of all the enzymes but, the levels were significantly decreased in increasing order DC+PE and DC+S+PE animals. Both SGPT and ALP levels were decreased in NC animals in the order NC+S+PE>NC+PE. However the levels of SGOT and ACP were increased in NC+PE and slightly decreased in NC+S+PE.

Urea and Creatinine (Figure 2 and 3): The serum levels of both urea and creatinine showed an identical trend of increasing decrease in PE and PE.S non

Fig. 1: Lipid peroxidation in liver, muscle and kidney of treated and control rats.

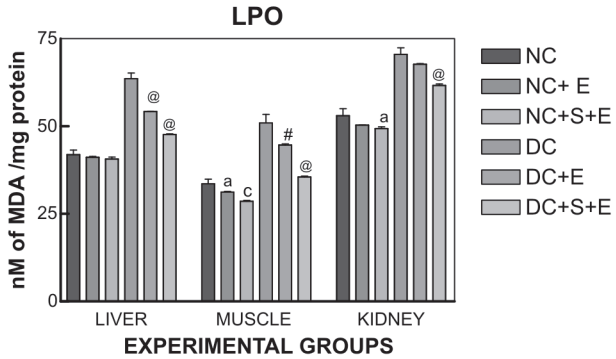

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Fig. 2: Serum Urea level in all the experimental groups.

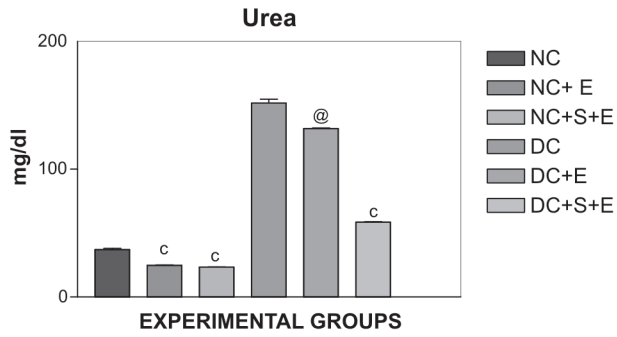

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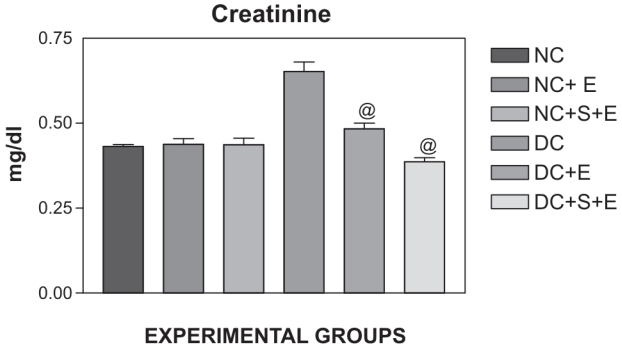
Fig. 3: Serum Creatinine level in all the experimental groups.



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diabetic and diabetic animals with DC animals having recorded generalised increase in the levels of both.

DISCUSSION

The present study, undertaken to evaluate the efficacy of a poly-herbal extract prepared of seven plants of common use or availability (PE) and a combination of PE and swimming exercise (S), has shown significant favorable effect of both in non-diabetic as well as diabetic rats.

The observations with reference to lipid metabolism show some differential effects in NC animals. Since there is tissue lipid depletion and increase in serum TG level in both NC.PE and NC.S+PE animals, it is inferable that increased lipid mobilization not balanced by utilization takes precedence when non-diabetic animals are either treated with PE or is subjected to swimming exercise along with treatment. Another differential effect is the decrease in hepatic and kidney cholesterol (but not in muscle which shows a paradoxical increase), and increase in serum cholesterol in NC animals treated with PE and, an increase in tissue cholesterol contents coupled with decrease in serum cholesterol level when subjected to a combination of S+PE. Apparently, PE increases tissue cholesterol breakdown while a combination of S+PE favors tissue cholesterol deposition in non-diabetic animals. However, the effect of both PE and S+PE is identical in DC animals as both tended to decrease tissue and serum lipids and cholesterol with the effect of S+PE being more pronounced. Clearly, both PE and S+PE (S+PE > PE) have a favorable antihyperlipidemic and antihypercholestrerolemic effects in diabetic animals. Since, hyperlipidemia and hypercholesterolemia are serious complications of diabetes responsible for secondary cardiovascular disorders, marked reduction in their levels brought about by PE is of high therapeutic merit and, an added exercise stint has added impact and reduces serum TG and Cholesterol levels maximally. It is presumable from this that, PE and S+PE promote lipid utilization and carbohydrate conservation and as such, *Murraya koenigii* [15], *Ocimum sanctum* [16], *Cassia fistula* [17] and *Annona sqamosa* [18] have all been shown to have some lipid and cholesterol lowering effects.

Growing evidence indicates enhanced oxidative stress in diabetic manifestations [19] and as such, the involvement of free radicals in induced Type I diabetic animal models has been shown [20]. Additionally, oxidative stress in diabetic conditions has also been deduced by an assessment of the status of scavenger enzymes, even though the collected data seem to be complicated due to organ and tissue specific activities. Alloxan or streptozotocin induced type I diabetic animal models have shown decrease in total SOD activity in almost all organs except for brain and lung [21]. Lipid peroxidation is found to be elevated in diabetic patients [1,22]. Increased lipid peroxidation and oxidative stress associated with diabetes have been attributed to the increased plasma glucose level [23]. The present study adequately demonstrates oxidative stress in diabetic animals as marked by the significantly elevated LPO levels, decreased activities of Catalase, SOD and GPx and decreased content of GSH in liver, muscle and kidney. Both PE and S+PE have significant antioxidative effects in both NC and DC animals. However, this effect is more pronounced in DC animals as seen by the near total amelioration of tissue LPO and significant reversal of compromised catalase and SOD activities and GSH content. In fact, GPx activity is not only reversed but attains significantly above non-diabetic levels. It is also worthwhile to know that, S+PE has maximal additive effect over that of PE. The active principles in the PE along with a bout of swimming exercise, have potent antioxidant activity by way of up-regulated expression of antioxidant enzymes and replenishment of tissue GSH pools. It is worth noting in this context, the statement of Johansson et al.[24] that "Given the number of short comings in clinical trials, it seems clear that more research on the use of antioxidants in the prevention of cardiovascular complications in diabetes is necessary and strongly encouraged. From a clinical viewpoint, however, efforts for the prevention of diabetic complications should seek to maximize the benefits of proven therapeutic strategies including appropriate life style changes and controlling blood pressure, blood glucose and lipids." In support of the presently observed decrease in oxidative stress brought about by PE, it is pertinent that, some of the plants used in our PE have been reported to exert certain degree of antioxidant effect. *Coccinia indica* [25], *Ocimum sanctum* 26], *Cassia fistula* [17, 27], *Mangifera indica* [28] and *Annona sqamosa* [29]. Apparently, PE combines in additive fashion the antioxidant properties of some of the constituent plants. Diabetes induced metabolic alterations and

oxidative stress are expected to cause certain degree of hepato-cellular damage and renal toxicity. The presently observed significantly elevated serum markers of hepatic (SGOT, SGPT, ALP, ACP) and renal (Urea and creatinine) functions is suggestive of Type I diabetes induced hepatic and renal damage. In general, both PE and S+PE show significant ameliorative effect in both NC and DC animals. Comparatively, the effect in DC animals is much pronounced and that too of the combination of S+PE. From the degree of reversal of the levels of serum markers of hepatic and renal function, it can be deduced that, PE has pronounced ameliorative effect on diabetes induced hepatic and renal stress and that, a regimen of swimming along with PE treatment is much more meaningful in over-coming functional impairment affecting liver and kidney. Pertinently, some of the constituent plants used for the preparation of PE have been demonstrated to have alleviating influence on diabetes induced hepatic and renal dysfunction: *Annona squamosa* [29, 30], *Murraya koenigii* [15,31], *Ocimum sanctum* [26], *Mangifera indica* [28] and *Cassia fistula* [32]. The decrease in corticosterone titre and increase in oestrogen levels brought about by PE and S+PE in diabetic animals are favorable hormonal *milieu* conducive for amelioration of oxidative stress and hepatic and renal dysfunctions. The report of Antus et al. [33] of estrogen exerting protective effect against development of glomerulosclerosis in the rat remnant kidney model, provides adequate justification for the purported hormonal *milieu*.

Overall, the present study provides support and evidence for consideration of a therapeutic approach combining the beneficial effects of a polyherbal preparation in association with adaptive physical activity for effective management of diabetic complications as suggested by the present observations of efficient lipid metabolism, oxidative stress and renal and hepatic dysfunction in Alloxan induced rat model of Type I diabetes.

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