Therapy with methanolic extract of *Pterocarpus marsupium* Roxb and *Ocimum sanctum* Linn reverses dyslipidemia and oxidative stress in alloxan induced type I diabetic rat model

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**Abstract**

Methanolic extracts of *Pterocarpus marsupium* Roxb (*P. marsupium*) and *Ocimum sanctum* Linn (*O. sanctum*) were prepared separately and then administered to both non-diabetic and alloxan induced diabetic adult female Wistar rats as a mixture of both at a dosage of 500 mg/kg body weight, and its effect was checked on serum and tissue lipids together with corticosterone, estrogen and progesterone profile. Further, tissue load of metabolites (cholesterol), enzymatic and non-enzymatic antioxidant status together with lipid peroxidation levels and serum markers of hepatic and renal damage were also assessed. Results of the present study strongly support the possibility of this herbal combination in humans to meet the objective of achieving a holistic amelioration and cure of diabetes as, the herbal extract mixture of *P. marsupium* and *O. sanctum* has succeeded in not only rectifying dyslipidemia but also in restoring the endogenous antioxidant levels to the pre diabetic status. Herbal preparations are ideal candidates of choice and in this context, the present combination of *P. marsupium* and *O. sanctum* provides compelling evidence for a holistic efficacy in amelioration of associated diabetic manifestations/dysregulations.

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**1. Introduction**

Prevalence of diabetes mellitus in Indian population is about 35 million as per a survey conducted by Ramachandran et al. (2008), with about 13 million of these cases assumed to go undiagnosed, of which around 50% cases are from rural and about 30% cases from urban areas of India. Recent findings suggest that, a disturbance in the pro-oxidant to anti-oxidant balance or, their homeostasis can add to the complications of almost all diseases. The increase in pro-oxidants is due to essentially an over production of free radicals and the consequent oxidative stress proving to be detrimental and, a number of theories have been suggested for the increase in pro-oxidants. Depletion in dietary and/or body antioxidants such as vitamin C (ascorbic acid – AA) and melatonin may contribute to poor and ineffective free radical scavenging activity and consequent oxidative stress (Dringen, 2000; Schulz et al., 2000).

It is well accepted that, the high oxidative stress in diabetics considerably contributes to the complication of this disease (Baynes, 1991; Baynes and Thorpe, 1999). Glucose oxidation is believed to be a major factor adding to the level of oxidative stress, as glucose is oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals (Wolf and Dean, 1987; Jiang et al., 1990). Non-enzymatic protein glycosylation associated with diabetes also has a role in diabetic complications.

*Pterocarpus marsupium* Roxb (*P. marsupium*), commonly known as Vijaysar is known for the treatment of diabetes since very long and it has some unique and unidentified features in protecting the pancreatic beta cells and their regeneration (Chakarvarty et al., 1981; Subramanian, 1981). The bioactive compounds of Vijaysar like (−) epicatechin (a flavonoid), marsupin (benzofuranone), and pterosupin (dihydrochalcone) have been shown to decrease blood glucose level in diabetics comparable to the effect of metformin (Marles and Farnsworth, 1995; Manickam et al., 1997).

Leaves of *Ocimum sanctum* Linn (*O. sanctum*), commonly known as Tulsi are similarly studied for their hypoglycaemic and antioxidative properties; it is shown to decrease blood glucose level in alloxan diabetic rats (Vats et al., 2002) but, most significant is the ability of Tulsi leaf extract to reduce lipid peroxidation and glutathione levels in Wistar rats (Jyoti et al., 2004). Leaves of Tulsi are...
very rich in oils, eugenol, euginal (phenolic compounds), ursolic acid (pentacyclic triterpene acid), cavacrol (monoterpenol phenol), limool (terpene alcohol) and sterols (Pattanayak et al., 2010) and, presence of eugenol helps in reducing the oxidative stress significantly (Uma Devi and Ganasonduri, 1999). A long study carried out by Halim et al. (2001), for eight weeks showed that, the aqueous extract of Tulsi leaves was a very effective antioxidant as, it could decrease the oxidative stress in circulating plasma and erythrocytes.

Since diabetic manifestations involve free radical associated damage, it has been hypothesized that, a combination of the two identified plants, P. marsupium (Vijaysar) and O. sanctum (Tulsi), can effectively target both, metabolic dysregulation and oxidative stress, associated with diabetic manifestations and be a better treatment paradigm than either alone.

2. Materials and methods

2.1. Preparation of extract (E)

The heart wood powder of P. marsupium (Vijaysar) bark was purchased from Sri. Gayatri Pharmaceuticals Private Limited, Rajpipla and fresh leaves of O. sanctum (Tulsi) were procured locally and authenticated by Prof. M. Daniel (Head, Department of Botany, M.S. University of Baroda). The leaves were shade dried, and ground in a mixer to get a fine powder. The fine powder of both the plants was extracted with HPLC grade methanol using a soxhelt (Borosil Glass Works, Mumbai, India) at boiling temperature (60°C) to 10 h separately; a dark brown coloured extract was obtained for Vijaysar whereas the extract obtained from Tulsi leaves was dark green in colour. These extracts were concentrated on rotavapour individually under reduced pressure and then dried to get a powder (Narendhirakannan et al., 2006). The dried powder obtained after this step from both the plants was collected in an air tight dark bottle separately and stored in a freezer at −80°C.

2.2. Experimental animals

Female Wistar rats (200–250 g) were housed in the departmental animal house under controlled room temperature (21 ± 2°C). 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, M.S. University of Baroda, Vadodara (Approval No. 827/ac/04/CP/CSEA), and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines were followed strictly.

2.3. Induction of Type I diabetes

Diabetes was induced in experimental rats by single intraperitoneal injection of alloxan (Diamond et al., 1989; Owu et al., 2006; Dhanabal et al., 2008) and the dosage (120 mg/kg body weight) was standardized for our animals as per the procedure described earlier (Singh et al., 2010). Briefly, the specific amount of alloxan (Sigma Chemicals, USA) was dissolved in saline freshly before administration and diluted in such a way that 0.5 ml contained the requisite concentration of alloxan.

2.4. Animal experiments

A total of 24 rats (12 normal and 12 diabetic rats) of Wistar strain as mentioned above were divided into four groups of six animals each. Group I consisted of normal non-diabetic rats (NC) which received vehicle alone while, Group II consisted of non-diabetic normal rats treated with a mixture of Vijaysar and Tulsi extract (E) at a dose of 500 mg/kg body weight; a dosage arrived at as maximally effective by standardization with different doses. Group III comprised of diabetic rats (DC) which received vehicle only while Group IV consisted of diabetic rats treated with 500 mg/kg body weight of extract mixture (E) seven days after alloxan administration. The animals received the scheduled treatment of vehicle (0.33% CMC) or extract mixture (E) by oral gavage for 15 days. Extract supplementation was started seven days after alloxanization and diabetic status was checked by blood glucose levels and animals with blood glucose levels of 300 mg/dl or more were considered diabetic and taken for experimentation.

2.5. Biochemical analysis

At the end of 15 days of treatment period, the rats were sacrificed by cervical dislocation after an overnight fast. Liver, Muscle and Kidney were excised out and stored at −80°C for further analysis. Lipid peroxidation (LPO) was determined as per the method described by Beuge and Aust (1978) while, Reduced glutathione (GlSH), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) were assayed by the methods of Beutler et al. (1963), Marklund and Marklund (1974), Sinha (1972) and Rotruck et al. (1973) respectively. Since works with the two plants individually have assessed effectiveness after 30 days of treatment and we were interested in checking the cumulative efficacy of the two together, assessment was done after 15 days of treatment.

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

(A) Corticosterone and progesterone (Immuno-Technology & Steroid Laboratory Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, Munirka, New Delhi).
(B) Estradiol (Biocheck Inc., California).
(C) Serum cholesterol (Accurex Biomedical Pvt Ltd.).
(D) Serum triglyceride (Accurex Biomedical Pvt Ltd.).
(E) HDL (Nicolas Piramal India Ltd.).
(F) SGPT (Agappe Diagnostics Ltd.).
(G) SGOT (Crest Biosystem Ltd.).
(H) Alkaline phosphatase (ALP) (Rekon Diagnostics Pvt Ltd.).
(I) Acid phosphatase (ACP) (Aspen Laboratories). Tissue cholesterol and lipids were assayed by the methods of Crawford (1959) and Folch et al. (1957) respectively.

2.6. 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. Serum hormone profile (Table 1)

Corticosterone (Cort), insulin, oestrogen (E2) and progesterone (P4).

Of the three hormones assayed, while oestrogen and progesterone did not show any significant change, corticosterone
was significantly reduced in extract treated non-diabetic animals. Diabetic animals showed significant decrease in corticosterone and progesterone levels while, oestrogen titre was significantly increased. Extract treated diabetic animals depicted a reversal of corticosterone and oestrogen titres towards non-diabetic levels, while, progesterone showed a further decrease.

3.2. Serum lipids (Table 2)

Except for an increase in serum triglycerides and VLDL levels of extract treated non-diabetic rats, there was no effect on any of the other serum cholesterol fractions. Diabetic rats showed significant increase in serum triglyceride and cholesterol fractions, the levels of which were significantly decreased on treatment with extract.

3.3. Tissue lipid and cholesterol (Figs. 1 and 2)

The extract showed a mild lipid and cholesterol lowering effect in non-diabetic rats, while the levels of both the metabolites were significantly decreased in diabetic rats. The extract treated diabetic rats have shown a significant retrieval in the levels of both the metabolites in all the tissues.

3.4. Oxidative stress parameters

3.4.1. Lipid peroxidation (LPO) (Fig. 3)

Diabetic animals showed significant increase in LPO in liver, muscle and kidney. While, extract treatment to non-diabetic animals had no significant effect, extract treated diabetic animals showed significant reduction towards non-diabetic levels.

3.4.2. Superoxide dismutase (SOD) (Fig. 4)

Diabetic animals showed a significant decrease in hepatic, muscle and renal SOD activity. Extract treatment of non-diabetic animals registered insignificant increase while, treatment of diabetic animals depicted significant increase.

3.4.3. Catalase (Fig. 5)

Diabetic animals showed significant decrease in hepatic, muscle and renal catalase activity. While extract treatment of non-diabetic rats did not show any remarkable effect, diabetic animals treated with extract showed significant increase towards non-diabetic levels, though still with a deficit.

3.4.4. Glutathion peroxidase (GPx) (Fig. 6)

Diabetic animals showed significant decrease in hepatic, muscle and renal GPx activity. Extract treatment increased the levels of enzyme activity, though still significantly lesser than non-diabetic levels. Extract treatment of non-diabetic rats showed no significant change.

Table 1
Serum hormone profile of extract treated non-diabetic and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Corticosterone (ng/ml)</th>
<th>Estradiol (pg/ml)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>8.95 ± 0.59</td>
<td>0.19 ± 0.002</td>
<td>66.05 ± 3.48</td>
</tr>
<tr>
<td>NC + E</td>
<td>6.30 ± 0.11</td>
<td>0.16 ± 0.001</td>
<td>61.90 ± 2.17</td>
</tr>
<tr>
<td>DC</td>
<td>25.0 ± 1.45</td>
<td>1.98 ± 0.0012</td>
<td>54.68 ± 1.74</td>
</tr>
<tr>
<td>DC + E</td>
<td>12.12 ± 1.21*</td>
<td>0.97 ± 0.003*</td>
<td>29.17 ± 2.60*</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE. NC = non-diabetic control, NC + E = non-diabetic control + extract, DC = diabetic control and DC + E = diabetic control + extract.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>CHO</th>
<th>TG</th>
<th>LDL</th>
<th>VLDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>80.00 ± 2.31</td>
<td>68.67 ± 3.44</td>
<td>15.00 ± 1.73</td>
<td>13.33 ± 1.73</td>
<td>50 ± 1.76</td>
</tr>
<tr>
<td>NC + E</td>
<td>71.33 ± 3.20*</td>
<td>98.21 ± 6.07*</td>
<td>10.33 ± 1.44*</td>
<td>11.33 ± 2.67*</td>
<td>50.00 ± 4.63*</td>
</tr>
<tr>
<td>DC</td>
<td>97.00 ± 4.33*</td>
<td>140.00 ± 2.33*</td>
<td>30.66 ± 0.80*</td>
<td>22.22 ± 2.89*</td>
<td>45.33 ± 2.60*</td>
</tr>
<tr>
<td>DC + E</td>
<td>69.66 ± 3.44*</td>
<td>78.33 ± 3.38*</td>
<td>6.66 ± 0.29*</td>
<td>15.5 ± 1.16*</td>
<td>47 ± 4.93*</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE. NC = non-diabetic control, NC + E = non-diabetic control + extract, DC = diabetic control and DC + E = diabetic control + extract.

* p < 0.01 compared to NC.
* p < 0.0005 compared to NC.
* p < 0.0025 compared to DC.
* p < 0.0005 compared to DC.
* p < 0.0005 compared to DC.

3.4.5. Reduced glutathione (GSH) (Fig. 7)
Diabetic animals showed significant decrease in hepatic, muscle and renal GSH contents. While, extract treatment to non-diabetic animals was marked by significant increase to above normal levels, extract treatment of diabetic animals showed an increase in GSH content in all the three tissues. In the extract treated diabetic rats, the hepatic GSH content was significantly increased while, muscle and renal GSH contents were reverted to normal levels.

3.4.6. Ascorbic acid (Fig. 8)
Diabetic animals showed significant decrease in hepatic, muscle and renal ascorbic acid contents. While, extract treatment of non-diabetic animals was without effect, diabetic animals treated with

Fig. 5. Tissue catalase activity of extract treated non-diabetic and diabetic rats. Data are expressed as Mean±SE; NC = non-diabetic control, NC+E = non-diabetic control + extract, DC = diabetic control and DC+E = diabetic control + extract; a) p<0.05, e) p<0.0005 compared to NC and *p<0.0005 compared to DC.

Fig. 6. Tissue glutathione peroxide activity of extract treated non-diabetic and diabetic rats. Data are expressed as Mean±SE; NC = non-diabetic control, NC+E = non-diabetic control + extract, DC = diabetic control and DC+E = diabetic control + extract; b) p<0.025, d) p<0.005 compared to NC.

Fig. 7. Tissue glutathione content of extract treated non-diabetic and diabetic rats. Data are expressed as Mean±SE; NC = non-diabetic control, NC+E = non-diabetic control + extract, DC = diabetic control and DC+E = diabetic control + extract; b) p<0.025, e) p<0.0005 compared to NC and ) p<0.005, °p<0.0005 compared to DC.

Fig. 8. Tissue Ascorbic acid content of extract treated non-diabetic and diabetic rats. Data are expressed as Mean±SE; NC = non-diabetic control, NC+E = non-diabetic control + extract, DC = diabetic control and DC+E = diabetic control + extract; c) p<0.01, e) p<0.0005 compared to NC and *p<0.0005 compared to DC.
Table 3
Serum markers of hepatic dysfunction in control and extract treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT (U/l)</th>
<th>SGOT (U/l)</th>
<th>ALP (U/l)</th>
<th>ACP (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>40.00 ± 4.04</td>
<td>70.00 ± 2.95</td>
<td>205.0 ± 2.648</td>
<td>8.5 ± 0.86</td>
</tr>
<tr>
<td>NC + E</td>
<td>37.00 ± 3.79</td>
<td>74.00 ± 1.76</td>
<td>198.0 ± 3.18</td>
<td>8.62 ± 0.18</td>
</tr>
<tr>
<td>DC</td>
<td>123.00 ± 5.86*</td>
<td>290.0 ± 5.78*</td>
<td>471.0 ± 2.33*</td>
<td>12.6 ± 0.614</td>
</tr>
<tr>
<td>DC + E</td>
<td>115.00 ± 5.93</td>
<td>192.0 ± 6.39*</td>
<td>287.0 ± 3.18*</td>
<td>10.5 ± 0.864</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE; NC = non-diabetic control, NC + E = non-diabetic control + extract, DC = diabetic control and DC + E = diabetic control + extract.

* p < 0.005 compared to NC.
* p < 0.0005 compared to NC.
* p < 0.005 compared to DC.
* p < 0.0005 compared to DC.

extract showed significant increase though not attaining the non-diabetic levels.

3.5. Serum markers of hepatic and renal function

3.5.1. SGPT and SGOT (Table 3)
The serum level of SGOT in non-diabetic animals was nearly double that of SGPT. Diabetic animals showed significant increase in the serum levels of both the enzymes and, extract treatment of diabetic animals decreased the levels of both, more so of SGOT, though the levels were still higher than in non-diabetic animals.

3.5.2. ALP and ACP (Table 3)
The serum level of ALP activity was significantly higher than that of ACP in non-diabetic animals. Diabetes tended to increase the levels of both the enzymes significantly, more so of ALP. Extract treatment of non-diabetic animals was of no consequence while, treatment of diabetic animals showed significant amelioration, more so of ALP.

3.5.3. Urea and creatinine (Figs. 9 and 10)
Diabetes was marked by significant increase in serum levels of urea and creatinine. Treatment of non-diabetic animals with extract was of no significant consequence but, treatment of diabetic animals tended to significantly minimize the levels of urea and creatinine though, still significantly higher than the non-diabetic levels.

4. Discussion
An earlier study had shown the efficacy of PM + OS in ameliorating diabetic glycemic dysregulation, hypoinsulinemia, insulin sensitivity and peripheral glucose uptake by upregulation of GLUT-4 (Singh et al., communicated). The present study in combination
tries to evaluate the efficacy of PM + OS in combating diabetes associated complications such as dyslipidemia, oxidative stress and hepatic and renal dysfunction. Moreover, the extract had no adverse effects as no hematological or behavioral changes were noted (data not shown).

Dyslipidemia is an essential accompaniment of diabetes. Whereas hypertriglyceridaemia and hypercholesterolaemia could be considered more of a consequence of Type I diabetes, the same could be a cause or consequence of Type 2 diabetes. Irrespective of cause or consequence, dyslipidemia is a diabetic manifestation and even in the present study, significant elevations are recorded for serum TG (103%), TC (21%), LDL (146%) and VLDL (115%) along with tissue (hepatic – 114%, muscle – 137%, kidney – 56%) cholesterol and lipid contents, in diabetic rats. Some of the previous studies have shown a better lipid lowering effect of PM extract or its constituent fraction (Jahroni and Ray, 1993; Grover et al., 2002; Dhanabal et al., 2006; Gayathri and Kannabiran, 2008) while, OS extract had been shown to exert minimal effect (Eshrat Halim and Mukhopadhyay, 2006). Since the higher tissue and serum lipids in diabetes contribute to many secondary complications affecting the cardiovascular system, it is essential for an antidiabetic prepara-
tion to have significant hypolipidemic and/or hypercholesterolemic effect. The present study on PM + OS combination extract in this context shows very potent effect in maintaining hepatic cholesterol content and serum triglycerides, VLDL and HDL levels to near normal non-diabetic state while, lowering serum cholesterol and LDL to even below the non-diabetic control levels. Muscle and kidney cholesterol contents showed a slow response though, the reduction from the diabetic high was to the tune of 50%. Clearly, a combination of PM and OS has the greatest lipid lowering potential and exerts corrective measures on the metabolic machinery responsible for diabetic dyslipidemia.

Similar to dyslipidemia, oxidative stress is another feature which can be both cause and consequence of diabetes and, persistent oxidative stress results in many secondary complications of serious nature. Enough evidence is available to show that, diabetic patients are under oxidative stress and that, increased oxidative stress contributes to the development and progression of diabetes and associated complications (Bonnefont-Rousselot et al., 2000; Maritim et al., 2003). Hyperglycaemia can, not only engender free radicals but, also impair endogenous antioxidant defence system (Saxena et al., 1993; Maritim et al., 2003). Compromised antioxidant system, denoted by increased lipid peroxidation and decreased levels of both non-enzymatic and enzymatic antioxidants, is a feature of diabetes and, such changes have been noted in alloxan or streptozotocin induced diabetes (Bonnefont-Rousselot et al., 2000; Maritim et al., 2003). In the present study, evaluation of oxidative stress in the form of levels of LPO, non-enzymatic (GSH) and enzymatic (SOD, Cat, and GPx) antioxidants in liver, muscle and kidney has depicted an increase in LPO and decrease in antioxidants ranging from 30% to 50% at an average, indicating co-existence of metabolic disturbances and oxidative stress in diabetic animals. Therefore, any treatment for diabetes should address to contain oxidative stress and restoring the antioxidant defense system apart from exerting glycaemic control. The desired effect of any anti-diabetic drug is no doubt normoglycaemia but apart from insulin, only a limited number of drugs such as melanatonin, probucol, vitamin C and E plus β Carotene and α-lipoic acid are capable of reversing the severe diabetic hyperglycaemia (Maritim et al., 2003). However, most of the antioxidants fail to achieve glucose regulation and hence the suggestion that they may be given as adjuvants to insulin therapy or hyperglycaemic anti diabetic drugs in vogue (Maritim et al., 2003). On the other hand, continuous treatment with insulin or hypoglycaemics though may help achieve a stable glycaemic state with some favourable effect on anti oxidant/oxidant balance, in reality, total normalization of antioxidant status seems very remote and not achieved (Sharma et al., 1998; Bonnefont-Rousselot et al., 2000). It is here, that, traditional herbal preparations could be of invaluable help. Of the two plants, PM and OS used in this study, though the former is more potent in glycemic and lipid regulation with only milder recovery effect on endogenous antioxidant levels (Joshi et al., 2004), the latter is more potent in reversing oxidative stress by normalizing antioxid-

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