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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.

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) and excessive production of free radicals is an observed phenomenon in association with diabetes (Baynes, 1991; Chang et al., 1993; Young et al., 1995; 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 (a 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

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very rich in oils, eugenol, euginal (phenolic compounds), ursolic acid (pentacyclic triterpene acid), cavvacrol (monoterpenol phenol), limelool (terpene alcohol) and sterols (Pattanayak et al., 2010) and, presence of eugenol helps in reducing the oxidative stress significantly (Uma Devi and Ganasoundri, 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) up 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 −20 °C; yield obtained was about 12% for Vijaysar and 2% for Tulsi. The powders of Vijaysar and Tulsi were diluted together in (0.33%) carboxy methyl cellulose (CMC) everyday prior to treatment schedule at a dose of 500 mg/kg body weight for both the plants. Of the three dosages (100 mg, 250 mg and 500 mg) evaluated, the highest dose was found to be maximally effective and hence used in the present study. In common practice, *P. marsupium* is used as a powdered bark available with pharmaceutical dealers or as overnight bark extract in water, while, Tulsi leaves are used as tea or even eaten raw.

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, 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.

2.3. Induction of Type I diabetes

Diabetes was induced in experimental rats by single intraperitonial 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 (GSH), 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 (E_2) and progesterone (P4).

Of the three hormones assayed, while oestrogen and progesterone did not show any significant change, corticosterone