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"PROTECTIVE EFFECTS OF *PIPER NIGRUM* AND *VINCA ROSEA* IN ALLOXAN INDUCED DIABETIC RATS"

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Abstract : In the present study aqueous extract of *Piper nigrum* seeds and *Vinca rosea* flowers were administered orally to alloxan induced diabetic rats once a day for 4 weeks. These treatments lead to significant lowering of blood sugar level and reduction in serum lipids. The levels of antioxidant enzymes, catalase and glutathione peroxidase decreased in alloxan induced diabetic rats however these levels returned to normal in insulin, *P. nigrum* and *V. rosea* treated rats. There was no significant difference in superoxide dismutase activity in all groups compared to controls. Lipid peroxidation levels were significantly higher in diabetic rats and it was slightly increased in insulin, *P. nigrum* and *V. rosea* treated rats as compared to control rat. These results suggest that oxidative stress plays a key role in diabetes, and treatment with *P. nigrum* and *V. rosea* are useful in controlling not only the glucose and lipid levels but these components may also be helpful in strengthening the antioxidants potential.

Key words : diabetes free radicals oxidative stress *Piper nigrum* *Vinca rosea*

INTRODUCTION

Oxygen free radicals (OFRs) and lipid peroxidation have been implicated in the pathogenesis of a large number of diseases such as diabetes, cancer, infectious diseases, atherosclerosis and in aging (1, 2). Mechanisms that contribute to the formation of free radicals in diabetes mellitus include not only increased non-enzymic and auto-oxidative glycosylation, but also metabolic stress resulting form changes in energy metabolism, the levels of inflammatory mediators, and the status of antioxidant defense systems (3). The evidence indicates that oxidative stress is increased in diabetes due to overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant defenses. Oxidative stress, as well as non-enzymic glycosylation, is now considered as a major factor contributing to the extent of chronic diabetic complications (2–5). Free radicals meet many of the criteria required for a role in the pathogenesis of diabetic syndrome (4). To control ROS, aerobic cells have developed their own defense systems, the antioxidant system, based on enzymic control which

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includes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (5, 6).

Traditionally, herbs are being used for the treatment of various conditions throughout the world; in India we have a rich history of the therapeutic usage of medicinal plants. Many herbal products have been described for the cure of diabetes mellitus. The antidiabetic properties of some plants like Bittergourd (*Momordica charantia),* Neem (*Azadirachta Indica),* Tulsi (*Ocimum Sanctum),* and Garlic (*Allium Sativum)* are well known in India. These herbal mixtures have been cited as dietary supplements and are quoted as especially useful for lowering the blood glucose level in diabetic patients (7–10). This study was designed to investigate the protective effect of two new herbal extracts namely *P. nigrum* and *V. rosea* in lowering the blood glucose level, lipid peroxidation, serum lipids and antioxidant enzymes status in alloxan induced diabetic rats.

METHODS

Plant material

Fresh flowers of *V. rosea* were collected from the institute campus, Aligarh and the seeds of *P. nigrum* from local market. Identification of the samples were done by using standard botanical monographs. They were further confirmed with the Dept. of Botany, AMU, Aligarh. Ten grams of the flowers and 10 gms of seeds were cleaned and soaked in 200 ml distilled water and allowed to stand overnight at 4°C. The soaked material was homogenized and centrifuged. The supernatant was concentrated in a lyophilizer and kept separately in airtight containers in a deep freeze (–4°C) until use.

Animals and diets

Male albino rats (Wistar Strain), obtained from the experimental Animal facility center, J.N. Medical College, A.M.U., were used for these experiments. They were housed in cages with screen bottoms of stainless steel. The cages were kept in a room maintained at 22±2°C at a relative humidity of about 55% and exposed to a 12 h light and 12 h dark cycle. They were than randomly divided in to 5 groups of 10 animals each, the animals were fed water and diet (Hindustan Lever Ltd., India) ad libitum for a period of 4 weeks. The animals were weighed periodically once a week and records of their growth was maintained. Animal experiments were carried out based on the Institutional animal ethics committee (IAEC) guidelines.

Induction of diabetes

Experimental diabetes was induced by using alloxan monohydrate following the method of Sochor et al (11). Rats were fasted for 12 hr and diabetes was induced by a single intraperitonial *(i.p)* injection of alloxan dissolved in a freshly prepared 0.15 M sodium acetate buffer (pH 4.5), at a dose of 200 mg/kg body weight.

Rats were divided into the following groups.

Group 1 : Control rats given only saline.

Group 2 : Alloxan induced diabetic rats (alloxan 200 mg/kg body weight).

Group 3 : Alloxan induced diabetic rats treated with insulin *(i.p.* injection of 2 units of protamine-zinc insulin).

Group 4 : Alloxan induced diabetic rats treated with *piper nigrum* (0.5 ml per rat/day).

Group 5 : Alloxan induced diabetic rats treated with *vinca rosea* (0.5 ml per rat/day).

Blood sampling

The rats were fasted over night and sacrificed under light anesthesia (ether inhalation) at the end of 4 weeks of treatment. Blood samples were collected from the jugular vein of treated rats. The samples were taken in to tubes with out anticoagulant. After keeping the samples at room temperature for 2 hr, the serum was isolated by centrifugation for 20 min at 3000 rpm and stored at –4°C until analysis was completed.

Preparation of liver homogenate

The liver was quickly removed, washed with ice-cold normal saline and weighed. It was then freed of fat by the methods of Folch et al (12), and was then homogenized in buffer containing 50 mM Mannitol, 2 mM Tris Hol pH 7 (10%) in a Potter Elvehjem homogenizer fitted with a polyteflon plunger at high speed. The homogenate was centrifuged at 25000 rpm for 20 min at 4°C. The supernatant fraction was used for the determination of antioxidant enzymes.

Estimation of glucose

It was estimated by glucose oxidase using the kit by Ranbaxy laboratories, N. Delhi, India.

Measurement of serum lipid profile

Serum total cholesterol (TC), high density lipoprotein (HDL-C), and triglycerides (TG) were estimated using standard kit of Ranbaxy laboratories. LDL-C was calculated by using Friedwald Formula (13).

Measurement of lipid peroxidation and antioxidants in liver homogenate

The rate of lipid peroxidation in the liver was determined by quantitative estimation of thiobarbituric acid-reactive substance (TBRAS) by the method of Gutteridge et al (14). The changes in the antioxidant defense system was determined by studying superoxide dismutase activity by the method of Markuland and Markuland (15). Catalase activity was assayed by the method of Aebi (16). Glutathione peroxidase activity was determined by the method of Lawrence et al (17). Protein contents in various samples was estimated by the method of Lowry et al (18).

All spectrophotometric measurements were carried out using a Camspec UV-Visible spectrophotometer (Camspec M330B, London, UK) with 1.0 ml quartz cuvettes with light path of 1.0 cm. All enzymes assays were performed at 25°C.

Statistical analysis

Statistical analysis of the data was followed by student's t-test. The difference was considered significant when $P<0.05$. Values are shown as means±SEM.

RESULTS

The blood glucose levels and body weights of control, alloxan induced diabetic and rats treated with insulin, *P. nigrum* and *V. rosea* are presented in Table I. Insulin treatment was given as a standard treatment.

Alloxan induced diabetic rats showed increase in blood glucose level more than 2 folds ($P<0.001$). The body weight of diabetic rats decreased by about 30%. The liver weight decreased in proportion to the body weight. Insulin and *V. rosea* improved the body weight of diabetic rats after 30 days of treatment where as *P. nigrum* showed no significant improvement in the weights Blood glucose levels also showed a reversal near to control values by treatment with insulin, *p. nigrum* and *V. rosea,* however the effect of *V. rosea* was closer to insulin, as compared to *P. nigrum.* Table II shows serum lipid profile. The values of TC and LDL-C returned to values nearing control group and HDL-C values were found to be higher only in the group treated with *P. nigrum* extract. This showed that treatment with *P. nigrum* and *V. rosea* significantly

TABLE I: Effect of treatment for 4 weeks with extract of *P. nigrum* and *V. rosea* on blood glucose and body weight of control, diabetic and diabetic treated rats.

Parameter	*Control*	*Diabetic*	*Diabetic+Insulin*	*Diabetic+P. Nigrum*	*Diebetic+V. Rosea*
Body weight (gm)	205±8.5	142±6.7[b]	198±10.2	175±8.0	184±11.0
Liver weight (gm)	7.50±0.19	4.32±0.26[c]	6.40±0.30[b]	5.80±0.60	6.11±0.41
Blood glucose mg/100 ml	102±6.0	270±13.0[a]	120±10.0	129±8.0	125±9.0

Values are mean±S.E.M of six separate determinations. Student's t-test P value calculated compared to control value are: a = $P<0.001$, b = $P<0.01$, c = $P<0.05$.

TABLE II: Effect of treatment for 4 weeks with extract of *P. nigrum* and *V. rosea* on serum lipid profile and LPO of control, diabetic and diabetic treated rats.

Parameter	*Control*	*Diabetic*	*Diabetic+Insulin*	*Diabetic+P. Nigrum*	*Diebetic+V. Rosea*
TC (mg/100 ml)	148.0±12.2	228.0±14.6[a]	165.5±11.2	172.0±6.2[c]	151.0±10.5
LDL-C (mg/100 ml)	80.4±7.89	140±8.6[b]	95.2±10.8	90.0±14.1	89.6±11.4
HDL-C (mg/100 ml)	52.7±8.1	46.8±2.7	48.2±4.6	58.7±6.7	49.7±3.2
LDL/HDL	1.52±0.11	3.00±0.21[a]	1.91±0.71	1.81±0.50	1.52±0.24
TG (mg/100 ml)	104±6.4	176.5±16.3[a]	138.6±10.7[c]	130.5±12.6[c]	123.2±14.4
LPO[1]	0.249±0.008	0.413±0.005[b]	0.265±0.007	0.281±0.006	0.294±0.006[c]

Values are mean±S.E.M of six separate determinations. Student's t-test P value calculated compared to control value are: a = $P<0.001$, b = $P<0.01$, c = $P<0.05$.

[1]nanomoles/mg protein

TABLE III: Effect of treatment for 4 weeks with extract of *P. nigrum* and *V. rosea* on catalase, super peroxide and glutathione peroxidase of control, diabetic and diabetic treated rats.

Parameter	*Control*	*Diabetic*	*Diabetic+Insulin*	*Diabetic+P. Nigrum*	*Diebetic+V. Rosea*
Catalase[1]	0.161±0.017	0.109±0.011[b]	0.138±0.019	0.145±0.015[c]	0.153±0.022[c]
Superoxide[2] dismutase	17.20±3.06	13.72±3.65[b]	15.10±2.24	15.68±3.64	16.24±2.20[c]
Glutathione peroxidase[3]	0.154±0.014	0.118±0.018[a]	0.131±0.070	0.139±0.010[b]	0.147±0.016[c]

Values are mean±S.E.M of six separate determinations. Student's t-test P value calculated compared to control value are: a = P<0.001, b = P<0.01, c = P<0.05.

[1]U/mg protein×10^3

[2, 3]U/mg protein

improved the lipid profile in diabetic animals.

The increases in the levels of LPO due to the effect of diabetes are shown in Table–II. The results obtained showed that lipids of the diabetic rats are vulnerable to peroxidation due to the increased oxidative stress during diabetes (P<0.01). Insulin, *P. nigrum* and *V. rosea* were all able to reverse the altered LPO damage. The antioxidate enzymes CAT, SOD and GPx activities were determined in the liver of diabetic and *P. nigrum* and *V. rosea* treated rats and it was compared with control groups. The lowest activity of CAT, SOD and GPx was seen in the diabetic rats. The treatment with insulin, *P. nigrum* and *V. rosea* normalized the altered antioxidant enzymes levels of liver, occurred due to diabetes.

DISCUSSION

Alloxan in addition to hyperglycemia induces degenerative changes in the tissue, along with other complications like cardiomyopathy, and nephropathy. These are also common complications of insulin dependent DM. This pathogenicity is believed to be due to oxidative damage of the tissues by OFRs (19–22). The present study showed that extract of the two herbal plants namely *P. nigrum* and *V. rosea* produce a marked decrease in blood glucose and serum lipids in alloxan induced diabetic rats and that liver antioxidant enzyme activities were decreased during diabetes along with a significant increase in the LPO levels. LPO plays an important role in aging, atherosclerosis and in a number of diabetic complications (23, 24). As diabetes and its complications are associated with free radical mediated cellular damage, (22) herbal hypoglycemic agents are administered to diabetic rats to assess their antioxidant potential. Our results show that *P. nigrum* and *V. rosea* not only have hypoglycemic activity which increase gradually and was observed to be maximum at the end of the study period i.e 4 weeks but these compounds also significantly control the LPO levels in diabetic rats.

There are reports of both increased, (24) decreased, (25) as well as unchanged (23) SOD activity in diabetic animals. We found a significant decrease in GPx and CAT activity as compared to control. This result is contrary to that reported by Yadav and

Aydin (26, 27). In our study, there was no change in SOD activity. This finding is in accordance with that of Kesavulu et al (23). The decrease in activities of CAT and GPx in liver after diabetes makes it more susceptible to oxidative damages by the action of oxygen free radicals. The decreased activity of antioxidant molecules along with elevated LPO levels in diabetic rats could probably be associated with decreased antioxidant defense potential. The reversal in their content following treatment may be due to decreased oxidative load.

The reversal of the oxidative damage shown as a measure of antioxidant enzymes with the antidiabetic compound used, namely insulin, *P. nigrum* and *V. rosea* indicates that they have possibly antioxidant properties which play a crucial role in the defense against OFRs. Thus *V. rosea* flower and *P. nigrum* seeds are not only similar to insulin in having a hypoglycemic effect they also check the antioxidant level. This is necessary and sufficient requirement for the control of the complications arising from glycation and glycooxidation of proteins and membranes. Further studies are required to investigate the specific components responsible for hypoglycemic effect of the two herbal extracts.

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