MODULATION OF OXIDATIVE STRESS PARAMETERS BY TREATMENT WITH PIPER BETLE LEAF IN STREPTOZOTOCIN INDUCED DIABETIC RATS

P. SANTHAKUMARI, A. PRAKASAM, K.V. PUGALENDI

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar-608 002. Tamilnadu

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ABSTRACT

Objectives: To study the effect of oral administration of Piper betle leaf powder suspension on lipid peroxidation and antioxidants in streptozotocin (STZ) diabetic rats.

Methods: Male Wistar strain rats were orally administered the leaf suspension of P. betle, (75 and 150 mg/kg body weight) for 30 days. Plasma and erythrocytes were separated out and the liver and kidney were homogenized in ice-cold buffer and the assays of thiobarbituric acid reactive substances (TBARS), hydroperoxides, glutathione (GSH), superoxide dismutase (SOD EC 1.11.1.1), catalase (CAT EC 1.11.1.6) and glutathione peroxidase (GPx EC 1.11.1.9) were performed in the supernatant obtained from liver and kidney of control and STZ diabetic rats. Plasma TBARS, hydroperoxides, ascorbic acid and alpha-tocopherol were measured.

Results: Oral administration of P. betle (75 and 150 mg/kg body weight) for 30 days resulted in a significant reduction in plasma thiobarbituric acid reactive substances (TBARS), hydroperoxides, alpha-tocopherol and significant improvement in glutathione, superoxide dismutase, catalase and glutathione peroxidase in the liver and kidney of STZ diabetic rats when compared with untreated diabetic rats. The antioxidant effect of P. betle at 75 mg/kg for 30 days was found to be comparable to glibenclamide in diabetic rats.

Conclusion: The leaf suspension of P. betle 75 mg/kg body weight showed significant antioxidant effects in STZ diabetic rats.

KEY WORDS
Antioxidants diabetes mellitus lipid peroxidation

INTRODUCTION

Since the time of Charaka and Susruta many herbal medicines in different oral formulations have been recommended for the treatment of diabetes mellitus (Madhumeha). Extracts of drugs from plant sources such as Allium sativum (garlic), Azadirachta indica (neem), Vinca rosea (nayantara), Gymnema sylvestra (meshashringre), Trigonella foenum graecum (fenugreek), Momordica charantia (bitter gourd), Ficus bengalensis (banyan), Eugenia jambolana (black berry), Ocimum sanctum (tulsi) and Eclipta alba (karichalankanni) are some of the plants reported to posses antihyperglycemic activity in experimental animals1-3.

Many unknown and lesser-known plants are used in folk and tribal medicinal practices in India. The medicinal values of these plants are not known much to the scientific world. P. betle (Family Piperaceae) is one such a plant of the several ingredients in a chew commonly known as ‘pan’. The betel plant is a slender, aromatic creeper, rooting at the nodes. The branches of the plant are swollen at the nodes. The plant has alternate, heart shaped, smooth, shining and long stalked leaves with pointed apex4. Medicinally P. betle is an aromatic, carminative, stimulant and astringent used as a preventive for worms and in snake bite4. Juice of the leaves is dropped into eyes in painful infection and night blindness. Essential oil from leaves of this plant has been used for
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the treatment of respiratory catarrhs and as antiseptic and the fruit is employed with honey as a remedy for cough. Preliminary investigation in our laboratory showed that oral administration of aqueous suspension of \textit{P. betle} at a dose of 75 mg/kg body weight showed better sugar reduction than 150 mg/kg body weight (unpublished data). Further, comprehensive search of literature revealed that very limited work has been carried out to explore the effect of \textit{P. betle} on lipid peroxidation and antioxidants status. Hence, the present study was undertaken to investigate the antioxidant potential of the medicinally important herb and the tissue lipid peroxidation status.

MATERIALS AND METHODS

\textbf{Plant material:} \textit{Piper betle}.Linn (Syn:Chavica betle Miq.) popularly known as "vetrelei" in Tamil, "Betel" in English and "Nagavalli" in Sanskrit was purchased from the local market, Cuddalore district, Tamil Nadu, India. The plant was authenticated by a Taxonomist in the Department of Botany, Annamalai University and herbarium specimens were submitted (AU.3898). The authenticated plant leaves were used for the preparation of extract.

\textbf{Preparation of plant material:} Fresh leaves were collected and air-dried in shade at room temperature. The dried leaves were powdered mechanically and sieved using a fine muslin cloth. The fine powdered leaves were kept separately in airtight containers until the time of use.

\textbf{Drugs and chemicals:} Streptozotocin (STZ) was obtained from Sigma chemical company. All other chemicals used were of analytical grade.

\textbf{Experimental animals:} Male albino Wistar rats (150-200 g) bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used in this study. The animals were fed on a pellet diet (Hindustan Lever, India) and water \textit{ad libitum}. The animals were maintained in their respective groups for 30 days. All studies were conducted in accordance with the National Institute of Health “Guide for the Care and Use of Laboratory Animals” and the experiments were carried out as per the Institutional Ethics Committee.

\textbf{Experimental induction of diabetes:} Adult (9 weeks old) male Wistar rats were made diabetic with an intraperitoneal injection of streptozotocin (STZ, 50 mg/kg body weight) dissolved in citrate buffer (0.1 M, pH 4.5). Streptozotocin injected animals exhibited massive glycosuria and hyperglycaemia within a few days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, 96 h after the injection with STZ. Albin rats with blood glucose level above 200 mg/dL were considered to be diabetic and were used in this experiment. Six rats were injected with 2% gum acacia alone that served as control.

\textbf{Experimental design:} After the induction of diabetes the rats were divided into six groups of six animals each.

- **Group I**: Control rats received vehicle solution (2% gum acacia)
- **Group II**: Diabetic control
- **Group III**: Normal rats received \textit{P. betle} (75 mg/kg body weight) leaf powder in 2% gum acacia
- **Group IV**: Diabetic rats given \textit{P. betle} leaf powder (75 mg/kg body weight) in 2% gum acacia
- **Group V**: Diabetic rats given \textit{P. betle} leaf powder (150 mg/kg body weight) in 2% gum acacia.
- **Group VI**: Diabetic rats received glibenclamide (600 µg/kg body weight) as aqueous solution.

The vehicle and drugs were administered orally by an intragastric tube daily for 30 days. After 30 days of treatment, the rats were fasted overnight and sacrificed by cervical decapitation. The plasma was separated for the estimation of thiobarbituric acid reactive substances (TBARS), hydroperoxides, ascorbic acid and \textit{α}-tocopherol and the liver and kidney were dissected out and washed with ice-cold saline immediately. A portion of the tissue was homogenized using a Potter Elvejeham homogenizer and the extract was used for the estimation of TBARS, hydroperoxides, glutathione superoxide dismutase (SOD, EC 1.11.1.1), catalase (CAT, EC 1.11.1.6) glutathione peroxidase (GPx EC 1.11.1.9) and tissue protein.
Table 1. Effect of *Piper betle* on TBARS, hydroperoxides, ascorbic acid and α-tocopherol in the plasma of normal and experimental animals after 30 days treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmoles/ml)</th>
<th>Hydroperoxides (nmoles/ml)</th>
<th>Ascorbic acid (mg/dL)</th>
<th>α-tocopherol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (2% gum acacia)</td>
<td>1.70±0.15</td>
<td>0.44±0.04</td>
<td>1.8±0.17</td>
<td>1.8±0.34</td>
</tr>
<tr>
<td>Normal + <em>Piper betle</em> (75 mg/kg)</td>
<td>1.68±0.22</td>
<td>0.43±0.01</td>
<td>1.7±0.10</td>
<td>1.6±0.30</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>3.76±0.50</td>
<td>0.78±0.08</td>
<td>0.7±0.08</td>
<td>3.1±0.30</td>
</tr>
<tr>
<td>Diabetic + <em>Piper betle</em> (75 mg/kg)</td>
<td>1.80±0.41</td>
<td>0.48±0.05</td>
<td>1.2±0.10</td>
<td>2.2±0.26</td>
</tr>
<tr>
<td>Diabetic + <em>Piper betle</em> (150 mg/kg)</td>
<td>2.17±0.61</td>
<td>0.56±0.04</td>
<td>1.1±0.10</td>
<td>2.3±0.47</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (600 μg/kg)</td>
<td>1.75±0.35</td>
<td>0.45±0.02</td>
<td>1.4±0.10</td>
<td>1.9±0.27</td>
</tr>
</tbody>
</table>

| F    | 4.12 | 3.86 | 6.44 | 7.22 |
| P    | <0.05| <0.05| <0.05| <0.05|

Values are mean±SD of 6 rats from each group. df=5, 30. Values with different superscripts (a, b, c and d) differ significantly with each other at P<0.05. (Duncan’s multiple range test).

**Statistical analysis:** Values were represented as mean±SD. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using Duncan’s multiple range test. P values <0.05 were considered significant.

**RESULTS**

Table 1 and 2 show the levels of TBARS, hydroperoxides, ascorbic acid and α-tocopherol in plasma, liver and kidney of control and experimental animals. A significant elevation in plasma and tissue TBARS, hydroperoxides were observed in diabetic rats when compared with control rats. There was a significant reduction in plasma ascorbic acid and elevated α-tocopherol in diabetic rats compared to control rats. Oral administration of *P. betle* (75 and 150 mg / kg body weight) significantly reversed these enzymes to near normal value. Rats administered with *P. betle* (75 mg/kg body weight) alone did not produce any significant alteration in plasma and tissue antioxidant parameters.

**DISCUSSION**

Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated fatty acids. Under physiologic conditions, low concentrations of lipid peroxides are found in tissues. Karpen et al. observed an elevated level of lipid peroxides in the plasma of diabetic rats and lipid peroxidation is one of the characteristic features of chronic diabetes. Lipid peroxide mediated tissue damage has been observed in the development of both type 1 and 2 diabetes. Nakakimura and Mizuno have reported that the concentration of lipid peroxides increases in the kidney of diabetic rats and an increased level of TBARS is an index of lipid peroxidation. Our results show that in diabetic control animals the levels of TBARS and hydroperoxides were high in plasma, liver and kidney, due to increased lipid peroxidation. In *P. betle* and glibenclamide treated diabetic rats, the TBARS and hydroperoxides levels were low in plasma, liver and...
Table 2. Effect of *Piper betle* on TBARS and hydroperoxides in the liver and kidney of normal and experimental animals after 30 days treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (mmoles/100 g tissue)</th>
<th>Hydroperoxides (mmoles/100 g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Normal (2% gum acacia)</td>
<td>0.76±0.09*</td>
<td>1.45±0.18*</td>
</tr>
<tr>
<td>Normal + <em>Piper betle</em> (75 mg/kg)</td>
<td>0.78±0.05*</td>
<td>1.57±0.11*</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>1.87±0.33*</td>
<td>2.70±0.40*</td>
</tr>
<tr>
<td>Diabetic + <em>Piper betle</em> (75 mg/kg)</td>
<td>0.87±0.04*</td>
<td>1.85±0.07*</td>
</tr>
<tr>
<td>Diabetic + <em>Piper betle</em> (150 mg/kg)</td>
<td>1.28±0.12*</td>
<td>2.10±0.15*</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (600 µg/kg)</td>
<td>0.83±0.04*</td>
<td>1.61±0.11*</td>
</tr>
</tbody>
</table>

Values are mean±SD of 6 rats from each group. df=5,30. Values with different superscripts (a, b, c and d) differ significantly with each other at P<0.05. (Duncan’s multiple range test).

Table 3. Effect of *Piper betle* on GSH, SOD, CAT and GPx in liver and kidney of normal and experimental animals after 30 days treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mg/100 g tissue)</th>
<th>SOD (Unit/mg protein)</th>
<th>Catalase (Unit/mg protein)</th>
<th>GPx (Unit/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Normal (2% gum acacia)</td>
<td>52.0±4.38*</td>
<td>33.7±6.02*</td>
<td>6.80±0.25*</td>
<td>16.9±1.38*</td>
</tr>
<tr>
<td>Normal + <em>Piper betle</em> (75 mg/kg)</td>
<td>50.6±4.0*</td>
<td>34.6±4.13*</td>
<td>6.91±0.22*</td>
<td>17.1±1.51*</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>28.0±3.38*</td>
<td>18.7±1.70*</td>
<td>3.41±0.09*</td>
<td>10.1±0.91*</td>
</tr>
<tr>
<td>Diabetic + <em>Piper betle</em> (75 mg/kg)</td>
<td>38.5±4.35*</td>
<td>24.0±2.26*</td>
<td>5.01±0.34*</td>
<td>14.6±1.32*</td>
</tr>
<tr>
<td>Diabetic + <em>Piper betle</em> (150 mg/kg)</td>
<td>32.6±4.45*</td>
<td>20.0±2.84*</td>
<td>5.94±0.20*</td>
<td>13.7±1.02*</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (600 µg/kg)</td>
<td>46.3±0.98*</td>
<td>30.6±1.96*</td>
<td>6.48±0.24*</td>
<td>15.9±1.37*</td>
</tr>
</tbody>
</table>

Values are mean±SD of 6 rats from each group. df=5,30. Values with different superscripts (a, b, c, d and e) differ significantly with each other at P<0.05. (Duncan’s multiple range test).
kidney, which may be due to the free radical scavenging action of active ingredients in *P. betle*. Choudhary and Kale have reported that *P. betle* extract inhibited the radiation induced lipid peroxidation process effectively and that could be attributed to its ability to scavenge free radicals involved in initiation and propagation steps.

The most important antioxidant in the cell membrane is $\alpha$-tocopherol. It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxyl radicals, thus protecting the cell structures against damage. Increased level of $\alpha$-tocopherol found in the STZ diabetic rats as compared with control rats in our study may be due to the release of membrane bound $\alpha$-tocopherol from damaged cell membrane since it is water insoluble. Takeneka et al. have reported increased levels of alpha tocopherol in the liver of diabetic rats in spite of increased susceptibility to oxidation.

SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O$_2^-$), which damages the membrane and biological structures. Catalase has been shown to be responsible for the detoxification of significant amounts of H$_2$O$_2$. SOD and catalase are the two major scavenging enzymes that remove the toxic free radicals in vivo. Reduced activities of SOD and catalase in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals (O$_2^-$) and hydrogen peroxide. *P. betle* and glibenclamide treated rats showed decreased lipid peroxidation that is associated with increased activity of SOD and CAT.

GPx catalyzes the reduction of H$_2$O$_2$ to H$_2$O and O$_2$ at the expense of GSH. GPx activity is also reduced in diabetic condition. This may be due to inactivation of the enzyme involved in disposal of oxygen species and also insufficient availability of GSH. The present study also observed the depleted levels of GSH in STZ diabetic rats and elevation of GPx after treatment with *Piper betle* leaf suspension and glibenclamide.

Our study corroborates the study of Choudhary and Kale who have indicated the protective effects of *P. betle* and its potential to elevate the antioxidant status in radiation induced lipid peroxidation.

The ability of *P. betle* on tissue lipid peroxidation and antioxidant status in diabetic animals has not been studied before. The result of this study shows that a daily administration of an aqueous suspension of *P. betle* for 30 days provides better antioxidant potential and protection against tissue lipid peroxidation.

REFERENCES


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Convener, PP Surya Kumari Prize,  
The Chief Editor-IJP,  
Department of Pharmacology,  
JIPMER, Pondicherry - 605 006. India