Water and Ethanol Extracts of *Piper nigrum* in Regulating Thyroid Function and Lipid Peroxidation in Mice

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Abstract

An investigation was made to evaluate the effects of water and ethanol extracts of *Piper nigrum* L. fruits in the alteration of the serum thyroid hormone concentrations and tissue lipid peroxidation in liver, the main target organ of many drugs. The alcohol extract, at a dose of 4.0 mg/kg for 15 days, caused thyrotoxicosis as evidenced by increased concentrations of thyroxine and triiodothyronine. A concomitant increase in hepatic lipid peroxidation with a decrease in superoxide dismutase and/or catalase activities also indicated its peroxidative effect. However, a trial with the aqueous extract did not exhibit any toxic effect either on thyroid or in liver functions. Rather, the latter extract appeared to be antithyroidic and antiperoxidative in nature as it could decrease serum thyroid hormone concentrations and hepatic lipid peroxidation, respectively. It is suggested that the aqueous extract of *Piper nigrum* may be preferred over the alcohol extract for therapeutic uses.

Key words: *Piper nigrum*, alcohol extract, aqueous extract, thyrotoxicosis, lipid peroxidation.

Introduction

*Piper nigrum* L. (commonly known as black pepper) (Piperaceae) is a climbing shrub, mostly cultivated in the hot and moist parts of the world including India. Its unripe fruit is used as culinary spice and condiment. As medicine, it is believed to cure sore throat, skin diseases, flatulence, arthritic and various gastric ailments (Sivarajan & Balachandran, 1994). Some reports on this plant have also indicated its toxic effects (Shwaireb et al., 1990; Piyachaturawat et al., 1995; Daware et al., 2000). In order to ascertain whether its effects are based on the type of preparation, in the present investigation a study was made with aqueous and alcohol extracts on the thyroid function of mice. A supporting thyroid dependent parameter, activity of glucose 6-phosphatase (G-6-Pase), was simultaneously evaluated in the liver. To reveal the toxic effects, if any, lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities have also been studied in the liver, the major site of drug metabolism.

Materials and methods

Chemicals

Diethylenetriamine penta-acetic acid, Tris, Sodium dodecyl sulphate and thiobarbituric acid (TBA) were supplied by E. Merck, Mumbai, India. Other chemicals were of reagent grade and were obtained from Loba Chemie, Mumbai, India.

Radioimmunoassay (RIA) kits for the estimation of total serum triiodothyronine (T3) and thyroxine (T4) were purchased from Bhabha Atomic Research Center (BARC), Mumbai, India.

Preparation of the extract

Dried unripe *P. nigrum* seeds of good quality were purchased from the local market, authenticated by the taxonomist of our department and a voucher specimen was kept in the herbarium. Seeds were finely powdered and then subjected to decoction. In brief, 100 g of dried powder was boiled in 500 ml of water for 30 min and cooled at room temperature. The supernatant was then filtered and vacuum dried at...
55–60°C (yield 15%). The extract was finally dissolved in double-distilled water for oral administration. Aqueous ethanol extraction was performed using 95% ethanol at 55–60°C in a Soxhlet apparatus. The yield of this extract was 10.8%. As the aqueous ethanol extract was not soluble in water, Tween 80 was added (1 : 1 : 2; extract : Tween 80 : water) to produce a fine suspension.

**Experimental design**

**Expt. I: Effects of water extract**

Adult Swiss albino male mice (2–3 months old), weighing 28 ± 2g were obtained from the departmental animal house and were acclimatized in temperature (27 ± 1°C) and light controlled (14h light and 10h dark) room with the provision of food (Gold Mohur mouse feed, Hindustan Lever Ltd.; Mumbai, India) and water *ad libitum*. Mice were divided into two groups of seven each. Group I receiving 0.1 ml of double distilled water served as control, while Group II, treated with the water extract of *Piper* fruits at the dose of 4mg/kg (taken from Shwaireb et al., 1990) was considered as the treated group. The drug and the vehicle were administered by gastric intubations between 11:00 and 12:00h of the day to avoid circadian interference and the treatment was continued for 15 days.

**Expt. II: Effects of ethanol extract**

A similar study was repeated considering the ethanol extract. However, control animals received the vehicle (0.1 ml of 10% Tween-80 to each animal every day) and the water, the suspending agent for the ethanol preparation. Other animals were tested intragastrically with the plant at the same dose of 4mg/kg and for the same duration (i.e., 15 days).

**Biochemical estimations**

For the evaluation of LPO, SOD and CAT activities, the liver was homogenized in 10% w/v ice cold phosphate buffer (0.1 M, pH 7.4) and the homogenate was centrifuged at 15,000 × g for 30 min. LPO was determined in the supernatant by the reaction of TBA with malondialdehyde (MDA), a product formed due to the peroxidation of lipids, following the method of Ohkawa et al. (1979) as routinely done in our laboratory (Panda & Kar, 2000a, 2001). However, mouse hormone-free serum was used in the preparation of standards.

**Results and discussion**

Results (Fig. 1) indicated that the administration of the *Piper nigrum* water extract significantly decreased the serum T₃ and T₄ concentrations in male mice (p < 0.05 and p < 0.01, respectively, as compared to their respective control values). However, a significant increase in the concentration of the two hormones and in hepatic G-6-Pase activity was observed (p < 0.05 for T₃; p < 0.001 for T₄; p < 0.01 for the third one) following the administrations of the ethanol extract.

These results clearly reveal both thyroid-inhibitory as well as thyroid-stimulatory activities of *Piper nigrum* fruit extract, depending on the nature of the preparation. In fact, the present study appears to be the first investigation on the comparative effects of ethanol and aqueous extracts of *P. nigrum* fruits on the modulation of thyroidal status of an animal model, which reveal the opposite effects of the two different preparations.

The ethanol extract stimulated the thyroid function leading to an increase in thyroid hormone concentrations (a state of thyrotoxicosis). Hepatic tissues also exhibited (Table 1) a toxic effect as evidenced by an increase in LPO (p < 0.001) and decrease in antioxidant enzymes viz. SOD and CAT activities (p < 0.01 and <0.001, respectively). Interestingly, water extract induced decrease in thyroid hormone concentration revealed the antithyroidal property of this extract. Furthermore, no hepatotoxic effect was noticed by water extract, which was rather found to be antiperoxidative as it could significantly decrease LPO (p < 0.01).

G-6-pase activity is very often directly related to the alterations in thyroid hormone levels (Anderson et al., 1977; Panda & Kar, 2000). In case of water extract, the activity of this enzyme exhibited a decreasing trend; whereas in ethanol extract it increased significantly, which further corroborate the extract-induced alterations in thyroid hormone concentrations.

Out of the two thyroid hormones, T₄ is synthesized primarily in thyroid gland and is directly secreted into the blood. The other thyroid hormone, T₃, is largely generated in the extra thyroidal organs, mainly in liver, by monodeiodination of T₄ (Ganong, 1995). Therefore, the increase and decrease in serum T₃ concentrations following the plant extract treatment could be due to the inhibition/promotion of thyroid gland and the changes in serum T₃ might be the result of the alteration in the deiodination of T₄ to T₃ (Chopra et al., 1978).
Figure 1. Effects of water and ethanol extracts of *Piper nigrum* fruits (0.4 mg/kg b.wt. of each) for 15 days on the changes in serum T<sub>3</sub> (ng/ml × 10<sup>3</sup>), T<sub>4</sub> (ng/ml) and hepatic G-6-Pase activity (µM of inorganic phosphate liberated/hr/mg protein ×100) in male mice. WE, Water extract; AE, Ethanol extract; a, p < 0.001; b, p < 0.01 and c, p < 0.05 compared to the respective control values.

Table 1. Effects of *Piper nigrum* water and ethanolic extracts (4 mg/kg b.wt. of each) for 15 days on the changes in hepatic LPO (nM of MDA formed/hr/mg protein), SOD (units/mg protein) and CAT (µM of H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein) activities in male mice.

<table>
<thead>
<tr>
<th>Treatment Parameters</th>
<th>Water Extract</th>
<th>Ethanol Extract</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>LPO</td>
<td>0.833 ± 0.083</td>
<td>0.465± ± 0.070</td>
</tr>
<tr>
<td>SOD</td>
<td>6.56 ± 0.474</td>
<td>6.67 ± 0.551</td>
</tr>
<tr>
<td>CAT</td>
<td>43.21 ± 1.27</td>
<td>44.07 ± 3.71</td>
</tr>
</tbody>
</table>

Data are mean ± SEM (n = 7). a, p < 0.001 and b, p < 0.01 as compared to the respective control values.

It may be emphasized that the ethanol extract was observed to be peroxidative in nature as LPO was increased, accompanied by a decrease in the activities of antioxidants SOD and CAT. Although most plant extracts have been reported to be antiperoxidative, some of our earlier reports (Panda & Kar, 1998, 2000b) do indicate the peroxidative nature of a few plant extracts as is presently seen in the ethanol preparation. Since thyrotoxicosis has also been reported to increase LPO and to impair the activity of antioxidant enzymes (Pereira et al., 1994), the increase in LPO by ethanol extract of *Piper* might be the secondary effects of drug-induced increase in thyroid hormones. Interestingly in the aqueous extract, hepatotoxicity was not observed; rather there was a decrease in LPO that indicated an antiperoxidative nature of the extract. Thus, the aqueous extract of *Piper nigrum* seems to act as an antithyroid as well as an antiperoxidative agent.

On the basis of the present findings it can be concluded that the aqueous extract of *Piper nigrum* appears to be hepatoprotective and inhibitory to thyroid function. Therefore, it may be considered for detailed investigation for the treatment of thyrotoxicosis/hyperthyroidism.

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References


