ABSTRACT

The leaves of *Piper betle* L. (Piperaceae) are widely chewed in Bangladesh as betel quid with or without tobacco. Chewing of leaves of the plant is advised by the folk medicinal practitioners of Bangladesh to alleviate pain (particularly toothache) and lowering of blood sugar, as well as aid the digestive process. The objective of this study was to scientifically evaluate the folk medicinal practitioner’s claims of the antihyperglycemic and antinociceptive properties of *Piper betle* leaves. Antihyperglycemic activity evaluation was conducted through oral glucose tolerance tests in glucose-loaded Swiss albino mice, while antinociceptive activity tests were performed in gastric pain models in Swiss albino mice, where gastric pain was induced by intraperitoneal administration of acetic acid. In antihyperglycemic activity tests, methanolic extract of leaves demonstrated dose-dependent and significant lowering of blood sugar in glucose-challenged mice. At extract doses of 50, 100, 200 and 400 mg per kg body weight, prior oral administration of the extract reduced blood sugar levels by 31.01, 34.38, 38.88 and 46.74%, respectively, as compared to control animals. A standard antihyperglycemic drug, glibenclamide, when orally administered at a dose of 10 mg per kg body weight lowered blood glucose levels by 46.07%. As such, the results strongly indicate that leaves of the plant possess potent antihyperglycemic properties. In antinociceptive activity tests, the methanolic extract of the leaves significantly and dose-dependently reduced the number of gastric writhings in gastric pain-induced mice. At doses of 50, 100, 200 and 400 mg extract per kg body weight, the percent reductions in writhings were, respectively, 47.00, 63.28, 69.40 and 71.48 as compared to control mice. The standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 51.04 and 67.32%, respectively. The extract, therefore, appears to be more potent than aspirin in alleviation of pain. Overall, the results validate the folk medicinal uses of the leaves of this plant and suggest that more scientific researches need to be carried out on isolation and identification of the relevant bioactive components present within the leaves of this plant.

Key words: *Piper betle*, antihyperglycemic, antinociceptive, Piperaceae

Introduction

*Piper betle* L. (Piperaceae) is a vine, which is presumed to have originated from South and South East Asia (India, Nepal, Bangladesh and Sri Lanka). Leaves of the plant are widely chewed in many of these countries as betel quid (with or without tobacco) and is said to give a mild stimulant effect. Folk medicinal practitioners (Kavirajes) of Bangladesh advise people to chew leaves of this plant, particularly after heavy meals to aid digestion. Additionally, Kavirajes also advise consuming the leaves through chewing to alleviate pain (particularly toothache) and to lower blood sugar. Many pharmacological effects have been attributed to the leaves like anti-ulcer, anti-platelet aggregation, anti-fertility, cardiotonic, anti-tumor, and anthelmintic (reviewed in Vikash et al., 2012). Traditional medicinal practices of the Indian sub-continent like Ayurveda use the leaves for treatment of halitosis, bronchitis and elephantiasis. The leaves are also used in Ayurveda as anthelmintic, aphrodisiac, carminative and laxative. The Unani traditional medicine considers the leaves as styptic and vulnerary.

Anti-diabetic activities of aqueous and ethanolic extracts of the leaves have been reported in normoglycemic and streptozotocin-induced diabetic rats (Arambewela et al., 2005). Oral administration of leaf...
suspension to streptozotocin-induced diabetic rats for 30 days have been reported to significant reduction in blood glucose and glycosylated hemoglobin levels (Santhakumari et al., 2006). Although any analgesic properties of leaves of this plant are yet to be thoroughly documented, enough anecdotal evidence exists in Bangladesh on the efficacy of chewing leaves for alleviation of toothache. Close monitoring of indigenous medicinal practices and anecdotal evidences have often led to the discovery of many important modern drugs (Balick and Cox, 1996; Cotton, 1996; Gilani and Rahman, 2005). As such, it is important to closely observe indigenous medicinal practices instead of dismissing them as mere quackery as is often done by allopathic medicinal practitioners.

Since close observation of indigenous medicinal practices played and is still playing a pivotal role in the discovery of newer and better medicines, we are conducting a two-pronged approach over a number of years. The first part of this approach involves ethnomedicinal surveys among traditional medicinal practitioners of both the mainstream community as well as various tribes of Bangladesh (Rahmatullah et al., 2009a-c; Rahmatullah et al., 2010a-g; Rahmatullah et al., 2011a,b; Rahmatullah et al., 2012a-d). In the second phase of this multi-dimensional study, we are screening various medicinal plants obtained from our ethnomedicinal survey data and screening whole plants or plant parts for various pharmacological activities. Primarily, our screening has concentrated on anti-diabetic, anti-cancer and antinociceptive effects in these plants (Anwar et al., 2010; Jahan et al., 2010; Khan et al., 2010; Mannan et al., 2010; Rahman et al., 2010; Rahmatullah et al., 2010h; Shoha et al., 2010; Ali et al., 2011; Barman et al., 2011; Hossan et al., 2011; Jahan et al., 2011; Rahman et al., 2011; Sutradhar et al., 2011). Diabetes is fast attaining epidemic status throughout the world and there are no total cures in allopathic medicine against this disease. Cancer also afflicts millions of human beings throughout the world every year, and only some forms of cancer can be cured, and then if detected early. Pain, arising from various physical or emotional factors, also afflicts millions of people throughout the world on a daily basis. As such, better medicines against these illnesses can prove to be beneficial for human beings. Since *Piper betle* leaves are used in folk medicine of Bangladesh for treatment of both pain as well as to lower blood sugar, the objective of this study was to evaluate the antihyperglycemic and antinociceptive potentials of leaves of this plant.

**Materials and Methods**

Leaves of *Piper betle* were collected from Dhaka district, Bangladesh during January, 2012. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Voucher specimen No. 37,519). The sliced and air-dried leaves of *Piper betle* were grounded into a fine powder and 95g of the powder was extracted with methanol (1:5, w/v) for 48 hours. The extract was evaporated to dryness. The final weight of the extract was 6.00g.

**Chemicals:**

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin, glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh.

**Animals:**

In the present study, Swiss albino mice (male), which weighed between 15-22 g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

**Antihyperglycemic activity:**

Glucose tolerance property of methanol extract of *Piper betle* leaves was determined as per the procedure previously described by Joy and Kuttan (1999) with minor modifications. In brief, fasted mice were grouped into six groups of six mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received methanol extract of *Piper betle* leaves at doses of 50, 100, 200 and 400 mg per kg body weight. Each mouse was weighed and doses adjusted accordingly prior to administration of vehicle, standard drug, and test samples. All substances were orally administered. Following a period of one hour, all mice were orally administered 2 g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method (Venkatesh et al., 2004).
Antinociceptive activity:

Antinociceptive activity of the methanol extract of *Piper betle* leaves was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into seven groups of mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspirin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanolic leaf extract of *Piper betle* at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

Statistical analysis:

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

Results and Discussion

In antihyperglycemic activity tests, the methanolic extract was observed to produce a dose-dependent and statistically significant lowering of glucose concentrations in blood of glucose-loaded mice. The percent reductions in blood glucose concentrations when the extract was administered at doses of 50, 100, 200 and 400 mg per kg body weight of mice were, respectively, 31.01, 34.38, 38.88 and 46.74 as compared to control mice, which did not receive any extract or antihyperglycemic drug. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight to mice, was observed to lower blood sugar by 46.07%, when compared to control mice (i.e. mice administered vehicle only). Thus the antihyperglycemic activity of the extract can be considered to be comparable to glibenclamide, at least at the highest dose of the extract tested, i.e. 400 mg per kg body weight. The results are shown in Table 1. It may be added that the observed results validate the folk medicinal use of the leaves for lowering blood sugar levels.

The observation reduction of blood sugar by the extract can be attributed to any of three mechanisms or a combination of the mechanisms. Any bio-active compound or compounds may lower blood sugar either by potentiating the pancreatic secretion of insulin or increasing the glucose uptake (Farjou et al., 1987; Nyunai et al., 2009). Alternately, a compound or compounds may inhibit glucose absorption in gut (Bhowmik et al., 2009). In either of these mechanisms or a combination of these mechanisms, the resultant effect will be reduction of sugar levels in the blood. In this preliminary screening on the antihyperglycemic activity of methanolic extract of *Piper betle* leaves, we have not explored the actual mechanism behind the reduction of blood sugar in glucose-loaded mice, but further experiments are on the way to elucidate the actual mechanism(s).

In antinociceptive activity evaluation experiments, the methanolic extract of leaves was observed to dose-dependently and significantly reduce the number of writhings in mice arising from gastric pain. At extract doses of 50, 100, 200 and 400 mg per kg body weight, the percent reductions in the number of writhings were, respectively, 47.00, 63.28, 69.40 and 71.48. By comparison, the percent reductions in the number of writhings observed on administration of a standard antinociceptive drug aspirin at doses of 200 and 400 mg per kg body weight were, respectively, 51.04 and 67.32. The results are shown in Table 2. Thus the highest dose of the extract tested, namely 400 mg per kg body weight showed higher antinociceptive activity than the highest dose of the standard antinociceptive drug tested, namely that of aspirin at 400 mg per kg body weight. Our observed results not only validates the folk medicinal use of the leaves for treatment of pain, but also suggests the presence of component(s) within the leaves with strong antinociceptive potential, and which further merits isolation and identification of such components.

Pain (analgesia) can be central or peripheral, and both central and peripheral analgesia can be detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005), as has been done in the present study. Prostaglandins are regarded to be responsible for the sensation of pain. Production of prostaglandins [mainly prostacyclines (PGI2) and prostaglandin- (PG-E)] has been shown to be responsible for excitation of Adelta-nerve fibers, leading to the sensation of pain (Reynolds, 1982; Rang and Dale, 2003). As such, the antinociceptive activity exhibited by crude methanolic extract of the leaves may be due to the extract’s ability to block any synthesis of prostaglandins. This, in turn, may be mediated through inhibition of cyclooxygenase and/or lipoxygenase activities. It is to be noted that a similar mechanism has been proposed for antinociceptive activity of *Ficus deltoidea* aqueous extract in acetic acid-induced gastric pain model (Sulaiman et al., 2008), and this may also be the mechanism operating in the present study.
The identity of the chemical component(s) present in methanolic extract of leaves of *Piper betle* responsible for the observed antihyperglycemic and antinociceptive effects as observed in the present study remains to be elucidated. Leaves of *Piper betle* are known to be rich in the presence of a number of chemicals with important pharmacological activities. Such chemicals include hydroxychavicol, chavibetol, piperbetol, arecoline, carvacrol, carvophyllene, piperitol, eugenol, isoegenol, allyl pyrocatechol, chavicol, safrole, anethole, betasitosterol, beta-sitosterol palmitate, dotriacontanoic acid, tritriacontane, piperine, piperlonguminine, chavibetol acetate, estragole, piperols A and B, and pyrocatechol diacetate (Zeng et al., 1997; Parmar et al., 1998; Ramji et al., 2002; Samy et al., 2005; Chang et al., 2007).

Hydroxychavicol can suppress cyclooxygenase production (Chang et al., 2007) and so can be responsible for the observed antinociceptive effects of the extract. Arecoline, another component of leaves, also reportedly demonstrated antinociceptive activity (Kharkevich and Nemirovsky, 1990), and so can account for the observed antinociceptive effects. The therapeutic potential of *Ocimum sanctum* L. (including anti-diabetic effects) has been largely attributed to eugenol (Prakash and Gupta, 2005), which compound is also present in *Piper betle* leaves. The anti-oxidant and anti-diabetic potential of beta-sitosterol, another component of leaves of *Piper betle* has been demonstrated (Gupta et al., 2011). However, whether these compounds were responsible for the observed antihyperglycemic and antinociceptive effects as observed in the present study remain to be elucidated. Further experiments are being done in our laboratory towards determining the role of these phytochemicals. Taken together, the results obtained in the present study strongly validates the folk medicinal use in Bangladesh of *Piper betle* leaves for alleviation of pain and lowering of blood sugar, and once again underlines the need to pay attention to indigenous medicinal practices towards discovery of newer and more efficacious drugs.

Table 1: Effect of methanol extract of *Piper betle* leaves on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Blood glucose level (mmol/l)</th>
<th>% lowering of blood glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>4.45 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide (Group 2)</td>
<td>10 mg</td>
<td>2.40 ± 0.35</td>
<td>46.07*</td>
</tr>
<tr>
<td><em>Piper betle</em> (Group 3)</td>
<td>50 mg</td>
<td>3.07 ± 0.46</td>
<td>31.01*</td>
</tr>
<tr>
<td><em>Piper betle</em> (Group 4)</td>
<td>100 mg</td>
<td>2.92 ± 0.27</td>
<td>34.38*</td>
</tr>
<tr>
<td><em>Piper betle</em> (Group 5)</td>
<td>200 mg</td>
<td>2.72 ± 0.24</td>
<td>38.88*</td>
</tr>
<tr>
<td><em>Piper betle</em> (Group 6)</td>
<td>400 mg</td>
<td>2.37 ± 0.14</td>
<td>46.74*</td>
</tr>
</tbody>
</table>

All administrations were made orally. Values represented as mean ± SEM, (n=6); *P < 0.05; significant compared to hyperglycemic control animals.

Table 2: Antinociceptive effect of crude methanol extract of *Piper betle* leaves in the acetic acid-induced gastric pain model mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of writhings</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>8.17 ± 0.79</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin (Group 2)</td>
<td>200 mg</td>
<td>4.00 ± 0.58</td>
<td>51.04*</td>
</tr>
<tr>
<td>Aspirin (Group 3)</td>
<td>400 mg</td>
<td>2.67 ± 0.88</td>
<td>67.32*</td>
</tr>
<tr>
<td><em>Piper betle</em> (Group 4)</td>
<td>50 mg</td>
<td>4.33 ± 0.99</td>
<td>47.00*</td>
</tr>
<tr>
<td><em>Piper betle</em> (Group 5)</td>
<td>100 mg</td>
<td>3.00 ± 0.97</td>
<td>63.28*</td>
</tr>
<tr>
<td><em>Piper betle</em> (Group 6)</td>
<td>200 mg</td>
<td>2.50 ± 0.123</td>
<td>69.40*</td>
</tr>
<tr>
<td><em>Piper betle</em> (Group 7)</td>
<td>400 mg</td>
<td>2.33 ± 0.67</td>
<td>71.48*</td>
</tr>
</tbody>
</table>

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=6); *P < 0.05; significant compared to control.

References


