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Antipyretic Activity of *Peperomia pellucida* Leaves in Rabbit

Alam KHAN[1], Moizur RAHMAN[2], Shariful ISLAM[2]

[1]Department of Pharmacy, University of Rajshahi, Rajshahi 6205, BANGLADESH

[2]Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi 6205, BANGLADESH

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Abstract: Antipyretic effects of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the leaves of *Peperomia pellucida* (Linn.) HBK (Fam. Piperaceae) were investigated. Intraperitoneal administration of boiled milk at a dose of 0.5 ml/kg body weight in albino rabbit leads to pyrexia. Intraperitoneal (i.p.) administration of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the leaves of *P. pellucida* at a dose of 80 mg/kg body weight significantly reduced the elevated body temperature of rabbit. This antipyretic effect has been compared with antipyretic effect of standard aspirin and the solvent used.

Key Words: *Peperomia pellucida*, leaves, antipyretic, rabbits, boiled milk, aspirin

Peperomia pellucida Yapraklarının Tavşanlar Üzerine Ateş Düşürücü Etkisi

Özet: *Peperomia pellucida* (Linn.) HBK (Fam. Piperaceae) yapraklarının etanol ekstraktlarının petrol eter ve etil asetatta çözünen fraksiyonlarının antipiretik (ateş düşürücü) etkileri araştırılmıştır. Kaynatılmış sütün periton kesesi içine 0,5 ml/kg vücüt ağırlığı dozunda uygulanması albino tavşanlarda yüksek ateşe neden olmaktadır. *P. pellucida* yapraklarının etanol ekstraktlarının petrol eter ve etil asetatta çözünen fraksiyonlarının periton kesesi içine 80 mg/kg vücüt ağırlığı dozunda uygulanması tavşanın artmış olan vücüt sıcaklığını belirgin olarak düşürmüştür. Bu ateş düşürücü etki standart aspirin ve kullanılan çözücünün ateş düşürücü etkisi ile karşılaştırılmıştır.

Anahtar Sözcükler: *Peperomia pellucida*, yapraklar, ateş düşürücü, tavşan, kaynamış süt, aspirin

Introduction

Peperomia pellucida (Linn.) HBK (Fam. Piperaceae), locally known as Luchi Pata, is an annual herb (1) that is widely distributed in many South American and Asian countries (2-5). The plant is refrigerant; its leaves have been used traditionally in the treatment of headache, fever, eczema, abdominal pains, and convulsions (1). Evaluations of antibacterial, antiinflammatory, and analgesic activity of *P. pellucida* were reported in literature (2, 6-7). Isolation of antifungal and anticancer constituents from this plant was also reported (8-9). Although the leaves of the plant is used traditionally in the treatment of pyrexia and its antipyretic related activities (such as antiinflammatory and analgesic activity) were found experimentally, its antipyretic potential has not been explored yet. In the present study an attempt has been made to establish the antipyretic effect of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the leaves of *Peperomia pellucida*.

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states (10). It is the body's natural function to create an environment where infectious agents or damaged tissues can not survive (10). Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediators (cytokines, such as interleukin 1β, α, β, and TNF-α), which increase the synthesis of prostaglandin E_2 (PgE_2) near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature (11). When body temperature becomes high, the temperature regulatory system, which is governed by a nervous feedback mechanism, dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV (12). Most of the antipyretic drugs inhibit

COX-2 expression to reduce the elevated body temperature by inhibiting PgE_2 biosynthesis (13). These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles. Natural COX-2 inhibitors have lower selectivity with fewer side effects (13). A natural antipyretic agent with reduced or no toxicity is therefore, essential. As *Peperomia pellucida* leaves are an old traditional medicament used in fever (1), it is hoped that leaves of this plant will provide a cost effective alternative antipyretic agent. Hence, the present study was designed to determine the antipyretic effect of petroleum ether and ethyl acetate soluble fractions of ethanol extract of *P. pellucida* leaves.

Materials and Methods

Plant materials

Leaves of the plant were collected from various part of Lakshmipur district of Bangladesh with the help of a local herbalist and identified by Prof. A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh, where its voucher specimen (No. KM6543) was deposited. The leaves were air-dried and ground into powder.

Preparation of petroleum ether and ethyl acetate fractions of ethanol extract

The powder materials (450 g) were extracted with ethanol (3 L) in a Soxhlet apparatus (Quickfit, England) (14). The extraction was continued for 72 h at 65 ºC. The extract was filtered through filter paper. The filtrate was concentrated under reduced pressure at 50 ºC in a rotary vacuum evaporator to afford a blackish green mass (32 g). This green mass was further extracted (15) with petroleum ether (3 × 50 ml), ethyl acetate (3 × 50 ml) and methanol (3 × 50 ml) and dried under reduced pressure to afford petroleum ether (9 g), ethyl acetate (7 g), and methanol fractions (0.5 g), respectively.

Preparation of sample and standard solutions

To prepare sample and standard solutions, 2.5% ethanol in distilled water (autoclaved) was used as a solvent. The sample solutions of petroleum ether and ethyl acetate fractions were prepared by dissolving each dried fraction in the solvent to obtain 60 and 120 mg per 2 ml solution. To facilitate dissolution, a few drops of Tween 80 were added. Each fraction was administered at a dose of 40 and 80 mg/kg body weight (16).

Aspirin as Disprin soluble tablet was obtained from a local market of Reckitt Benckiser (Bangladesh) Ltd and used as an antipyretic agent. The standard solution was prepared by dissolving the tablet in the solvent to obtain 15 mg aspirin per 2 ml solution. The dose of aspirin was maintained at 10 mg/kg body weight (17).

Animals

The experiment was carried out on albino rabbits. They were 13-15 months old, of both sexes, weighing between 1.5 and 1.6 kg (18). They were collected from the International Center for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B). Considering the group, the rabbits were kept in iron cages (19) to adjust to the environment, and fed with cauliflower, cabbage, banana, and tap water for 40 days before the experiment. Food and water were withdrawn 6 h prior to the experiment (17). The animals were grouped as:

a. Experimental groups: 4 groups; 2 groups receiving petroleum ether fraction (2 doses; 40 and 80 mg/kg) and the other 2 groups receiving ethyl acetate fraction (2 doses; 40 and 80 mg/kg).

b. Control groups were:

i. Aspirin group (+Ve Control): Receiving standard antipyretic agent aspirin.

ii. Solvent group (-Ve Control): receiving solvent (used).

Number of rabbits in each group was 6.

Acute toxicity study

Acute toxicity study was carried out by graded doses of each fraction in albino mice. Both petroleum ether and ethyl acetate fractions were administered intraperitoneally in graded doses (200 to 1000 mg/kg body weight). They were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality (20).

Treatment protocol

Before the experiment, rectal temperatures of the rabbits were recorded by inserting a well lubricated bulb of a thermometer in to the rectum. Care was taken to insert it to the same depth each time (about 6 cm) (17). Milk was collected from local cattle. Rabbits were injected with boiled milk at room temperature at the dose of 0.5

ml/kg body weight to induce pyrexia. Induction of fever took about 1 to 2 h (17, 21).

Then the solvent (2 ml) was given on the negative control group, the known antipyretic agent aspirin solution (2 ml) was given on the positive control group and each sample solution (2 ml) was given to the corresponding experimental group (Table). Intraperitoneal route was used to administer boiled milk, aspirin solution, solvent, and sample solutions. Finally, rectal temperatures were recorded at 1 h intervals up to 3 h.

Statistical analysis

Data were presented as mean ± standard error (Mean ± SE). Student's t-test was used for comparison between the experimental and control groups. $P < 0.05$ was considered to be statistically significant.

Results

In acute toxicity study, both fractions were found to be safe and no mortality was observed to a dose as high as 1000 mg/kg. The resultant effects of both fractions of *Peperomia pellucida* leaves on boiled milk induced pyrexia in rabbits are depicted in Table. At a dose of 40 mg/kg body weight, petroleum ether and ethyl acetate fractions reduced 46.8 ± 0.35% and 40.0 ± 1.02%, respectively, of elevated rectal temperature compared to aspirin (92.5 ± 1.52% after 3 h). At a dose of 80 mg/kg body weight, petroleum ether and ethyl acetate fractions reduced 90.0 ± 0.63% and 70.0 ± 1.21%, respectively, of elevated rectal temperature compared to aspirin (92.5 ± 1.52% after 3 h). Thus both petroleum ether and ethyl acetate fractions produced significant ($P < 0.05$) antipyretic effect. It was also observed that the solvent have no effect on the reduction of pyrexia of rabbit.

Table. Effect of petroleum ether and ethyl acetate fractions of *Peperomia pellucida* leaves on boiled milk induced pyrexia in rabbit.

Groups	Dose	Rectal temperature (ºC)		Rectal temperature after treatment (ºC)		
		Normal (A)	3 h after boiled milk admin. (B)	1 h (C1)	2 h (C2)	3 h (C3)
Solvent	2 ml/rabbit	38.44 ± 0.31	40.16 ± 0.19	40.05 ± 0.12 (6.4 ± 0.27)	40.00 ± 0.54 (9.3 ± 0.12)	39.88 ± 0.07 (16.3 ± 0.74)
Aspirin	10 mg/kg	38.61 ± 0.14	40.11 ± 0.31	39.61 ± 0.32 (33.3 ± 0.13)*	38.72 ± 0.56 (92.6 ± 0.71)*	38.72 ± 0.62 (92.6 ± 1.52)*
Pet. ether	80 mg/kg	38.50 ± 0.09	40.16 ± 0.17	39.61 ± 0.32 (33.1 ± 0.51)*	38.72 ± 0.35 (86.7 ± 0.64)*	38.66 ± 0.52 (90.4 ± 0.65)*
Pet. ether	40 mg/kg	38.55 ± 0.09	40.33 ± 0.40	40.05 ± 0.19 (15.7 ± 0.15)	39.61 ± 0.22 (40. 4 ± 0.35)*	39.5 ± 0.37 (46.6 ± 0.34)*
Ethyl acetate	80 mg/kg	38.66 ± 0.17	40.33 ± 0.42	39.83 ± 0.30 (29.9 ± 0.31)	39.16 ± 0.22 (70.1 ± 0.74)*	39.16 ± 0.62 (70.1 ± 1.21)*
Ethyl acetate	40 mg/kg	38.77 ± 0.54	40.16 ± 0.28	39.88 ± 0.18 (20.1 ± 0.22)	39.61 ± 0.47 (39.6 ± 0.60)*	39.61 ± 0.52 (39.6 ± 1.01)*

All values are expressed as mean ± SE (n = 6), percentage reduction in rectal temperature is given within parentheses. * $P < 0.05$ significant compared to control (solvent).

$$\% \text{ reduction} = \frac{B - Cn}{B - A} \times 100; \text{ where } n = 1, 2 \text{ and } 3.$$

Discussion

The acute toxicity study result reveals that this plant might be considered a broad nontoxic one. Our experimental results exhibited that both petroleum ether and ethyl acetate fractions of ethanol extract of *Peperomia pellucida* leaves possess a significant antipyretic effect in the maintaining of normal body temperature and reduce boiled milk induced elevated rectal temperature in rabbits and their effect are comparable to that of standard antipyretic drug, aspirin. Such reduction of rectal temperature of the tested animals by both of the fractions at 80 mg/kg appears to be due to the presence of a single bioactive substance or a mixture of compounds in them. The isolation of beta sitosterol from *Peperomia pellucida* leaves of was reported in literature (1). The beta sitosterol is a plasminogen activator and promotes the formation of essential polyunsaturated fatty acids from linoleic acid, but linoleic acid is required for prostaglandin and leukotriene synthesis (22) and thus beta sitosterol reduces prostaglandin and leukotriene synthesis. Beta sitosterol possesses potent antiinflammatory and antipyretic activity (23) by reducing the secretion of proinflammatory cytokines and alpha-TNF (23-24). These phytosterols can enhance adaptive immunity through the stimulation of innate immune system termed as the 'adaptogen', which promotes overall health without side effects (25). It is also evident from the study that the antipyretic activity of petroleum ether fraction at 80 mg/kg body weight is almost similar to the standard aspirin group and is more active than the ethyl acetate fraction. The present study, therefore, supports the claims of traditional medicine practitioners as an antipyretic remedy. However, to know the exact mechanism of the action of *Peperomia pellucida* leaves extract, further study with purified fractions/bioactive compounds are needed.

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Corresponding author:
Alam KHAN
Department of Pharmacy,
University of Rajshahi,
Rajshahi- 6205,
BANGLADESH
E-mail: alamkhan792002@yahoo.co.in

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