Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading doi: 10.1016/S2222-1808(14)60760-X

Sedative, membrane stability, cytotoxic and antioxidant properties of methanol extract of leaves of *Protium serratum* Wall.

Md. Rafikul Islam[1*], Mohammad Fazlul Kabir[1], Rashedul Alam[1], Rana Dhar[1], Mohammad Nasiruddin Rana[1], Md. Ekramul Islam[2], Mst. Shahnaj Parvin[2], Md. Aslam Hossain[3]

[1]*Department of Pharmacy, International Islamic University, Chittagong, Bangladesh*

[2]*Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh*

[3]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh*

ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form 17 Jun 2014
Accepted 20 Aug 2014
Available online 22 Aug 2014

Keywords:
Protium serratum
Sedative
Membrane stability
Cytotoxicity
Antioxidant

ABSTRACT

Objective: To study the sedative, membrane stability, cytotoxic and antioxidant properties of the leaves of *Protium serratum* extracted using methanol.

Methods: Sedative test was performed using hole cross and open field methods at 200 and 400 mg/kg. Membrane stability of red blood cell was used for anti-inflammatory test at different concentrations. Cytotoxic study was performed using brine shrimp lethality test. Total flavonoid contents, total phenol contents and reducing power were used to assess antioxidant properties of the extract.

Results: Extract showed better sedative action at lower doses in both experiments. Maximum 73.33% locomotion reduction was found at 200 mg/kg at 120 min and that was 89.29% for diazepam in hole cross test. In membrane stability test, extract and standard drug diclofenac have 35.66% and 91.20% stability, respectively. LC_{50} value of the extract was 22.91 μg/mL. Total phenol and flavonoid contents were (55.53±14.63) mg gallic acid equivalent per gram of extract and (106.33±7.35) mg of quercetin equivalent per gram of extract, respectively per gram of extract. Significant reducing power was observed as compared to ascorbic acid.

Conclusions: Extract possesses good pharmacological properties. Hence, further extensive study is essential to find out possible active constituents for the treatment of anxiety, inflammation or sickle cell disease, cancer and free radical mediated abnormalities.

1. Introduction

Plants play very vital roles in our daily life. In earth, plants are known in pharmacy. People are using various kinds of plants before thousands years ago for the treatment of ailments. The World Health Organization estimates that 80% of African and Asian populations use traditional medicine as the first source for their health care needs[1]. Many modern drugs which are derived directly or indirectly from the medicinal plants are now used successfully. High class ingredients are preferred in medicinal plants which can be used as a drug development and drug synthesis. Plants contain diverse phytoconstituents and they possess distinct and unique properties. So, variant chemical structures and pharmacological actions of the plant constituents help us to find out effective drugs.

Protium serratum (Wall. ex Colebr.) Engl. (*P. serratum*) is a genus of more than 140 species of flowering plants in the family Burseraceae. The synonym of the plant is *Bursera serrata*. It is known as Chitrika, Hiliabhadi, Gutgutya, Neul and Neuor in Bengali. The local name of *P. serratum* is Gutgutiya (Chakma), Shu Dui Shi (Marma), Thai Cherem (Tripura). It is native in Bangladesh, Assam and Philippines. This large, evergreen and perennial tree found in the hill tracks of Bangladesh. Leaf blades are oblong, oblong-lanceolate, acuminate. Flowers are green and drupes are bright pink. The mature fruit is edible. They are also used in incense and perfumes. Bark paste is applied on boils and scabies[2]. Most of the members

*Corresponding author: Md. Rafikul Islam, Assistant Professor, Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, 154/A, College Road, Chittagong-4203, Bangladesh.
Tel: +88-01719-452356
E-mail: rafik_ruph@yahoo.com
Foundation Project: The total research work was supported by Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

of Burseraceae are known for their aromatic resins or gums and turpentine[3]. Turpentine compounds have sedative and anti-inflammatory effects. Some species of the Burseraceae family reported to have pharmacological properties. Hexane extracts of *Bursera simaruba* (L.) Sarg. leaves display anti-inflammatory activity on the adjuvant-carrageenan-induced inflammation in rats[4]. *Bursera schlechtendalii* (Burseraceae) has shown antitumor activity against the 9KB (adenocarcinoma of nasal pharynx) test system[5]. The extract of the plant *Bursera fagaroides* contains 3 compounds, which apparently are glycosides with a potent activity upon agglutination -immobilization and a low effect upon spermatocytes viability, which might be used as contraceptives[6]. Aqueous methanol extract of the leaves and fruits of the plant possess antioxidant activity[7]. Rashid *et al.*, isolated two terpenoids, β-amyrin (1) and β-sitostenone (2) and a coumarin, scopoletin (3) from petroleum ether and dichloromethane soluble extracts of the stem bark of *P. serratum* Wall. and dichloromethane extract was also reported to have antimicrobial and cytotoxic activity[8].

β-Amyrin, β-sitostenone and scopoletin present in *P. serratum* also isolated from different plant sources and possessed diverse pharmacologic actions. The mixture of α- and β-amyrin, pentacyclic triterpenes isolated from the stem bark resin of *Protium heptaphyllum* evidenced sedative and anxiolytic effects that might involve an action on benzodiazepine-type receptors and also an antidepressant effect where noradrenergic mechanisms will probably play a role[9]. β-Amyrin palmitate isolated from the leaves of *Lobelia inflata* possesses sedative action[10]. α and β-Amyrin retard acute inflammation in rat model of periodontitis[11]. *Quassia amara* (Family: Simaroubaceae) was reported to have antinociceptive, anti-inflammatory, muscle-relaxant, and sedative effects in rats and mice, and β-sitostenone, β-sitosterol, β-carbolines, β-dehydroquassins were main phytochemicals in it[12,13]. Antidepressant-like effect of scopoletin, a coumarin from *Polygala sabulosa* is dependent on the serotonergic [5-HT(2A) receptors], noradrenergic [(α1)- and (α2)-adrenoceptors] and dopaminergic [dopamine D(1) and D(2) receptors] systems[14]. Scopoletin shows anti-inflammatory activities through inhibition of eicosanoid biosynthesis, cell influx, and peroxidation[15]. *P. serratum* is medicinally important and contains active constituents (terpenoids and coumarin), so as a part of our ongoing research with medicinal plants from Bangladesh, we investigated sedative, membrane stability, cytotoxic and antioxidant activities of methanol extract of the plant.

2. Materials and methods

2.1. Plant material

The plant *P. serratum* was collected from Cox's Bazar, Chittagong, Bangladesh in 2013. The plant was identified by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh. A voucher specimen has been retained in the Chittagong Forestry with accession number 38015.

2.2. Preparation of extract

The collected plants were washed thoroughly with water, chopped and then dried under shade. The leaves were heated at 35 °C for 1 h and pulverized in a mechanical grinder. The powder obtained was successively extracted in methanol. The extract was made to dry by using rotary evaporator under reduced pressure.

2.3. Animals

Swiss albino mice of either sex having weight 25-30 g were collected from International Centre for Diarrhoeal Disease and Research, Bangladesh. The animals were housed under standard laboratory conditions [relative humidity 55%-65%, room temperature (23.0±2.0) °C and 12 h light: dark cycle] and acclimatized for 7 d. The animals were fed with standard diet and water.

2.4. Sedative activity

2.4.1. Hole cross test

The test was carried out as described by Takagi *et al*[16]. Hole cross apparatus is a wooden box having a dimensions of 30 cm×20 cm×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of a partitioning wall in the cage. Each mouse was immediately placed on one side of the cage after oral administration of test drugs. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min.

2.4.2. Open field test

The experiment was performed according to the methods described by Gupta *et al*[17]. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the mice was counted for 3 min on 0, 30, 60 and 120 min during the study period.

2.4.3. Membrane stability test

The test was performed with the approval of Ethics Committee of Pharmacy Department, International Islamic University Chittagong (approval No. ECPD-IIUC2013/09). Fresh blood was collected from healthy human volunteer under supervision of a physician. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at