IJPSR (2011), Vol. 2, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 May, 2011; received in revised form 21 August, 2011; accepted 23 August, 2011

PHARMACOLOGICAL STUDY OF THE *PELTOPHORUM PTEROCARPUM* FLOWER

M. Saiful Islam [1], Ronok Zahan [2], M. Badrul Alam [2], Marufa Naznin [3], M. A. Mosaddik [4], M. Ekramul Haque*[4]

Department of Pharmacy, International Islamic University [1], Chittagong, Bangladesh
Department of Pharmacy, Atish Dipankar Unversity of Science & Technology [2], Dhaka, Bangladesh
Department of Environmental Science, Independent University [3], Bangladesh
Department of Pharmacy, University of Rajshahi [4], Rajshahi-6205, Bangladesh

Keywords:

Peltophorum pterocarpum, Antibacterial, Ehrlich ascites carcinoma (EAC) cells, Analgesic Activity

Correspondence to Author:

Professor Md. Ekramul Haque

PhD, Department of Pharmacy, University of Rajshahi, Rajshahi - 6205, Bangladesh

ABSTRACT

The present study was designed to investigate *in vivo* analgesic and cytotoxicity as well as *in vitro* antibacterial activities of the methanol: ethylacetate (1: 9) extract of the flowers of *Peltophorum pterocarpum.* Analgesic activity was assayed by acetic acid induced assay method wherein the extract showed moderate activity in a dose dependent manner. In respect of *in vivo* cytotoxicity, the flower extract of *Peltophorum pterocarpum* showed moderate activity with 30% cell growth inhibition against Ehrlich Ascites Carcinoma (EAC) cells at a dose of 50mg/kg. Moreover, Antibacterial test were done against five Gram-positive and four Gram-negative bacteria. Flower extract was active against all tested microbial species except *Staphylococcus aureus* and the zone of inhibition range was found to be 12±0.32 to 18±0.11 mm.

INTRODUCTION: Medicinal plants from time immemorial have been used in virtually all cultures as a source of medicine [1]. They are considered as the backbone of traditional medicine and are widely used to treat a plethora of acute and chronic diseases ranging from the common cold to complex human diseases all over the world. Due to the shortcomings of currently available drugs there has been continuing interests in the study of medicinal plants for discovery of new pharmacotherapeutic agents [2].

Bangladesh is an attractive repository of various medicinal plants [3]. Thus, exploring such abundant natural gifts, hundreds of traditional medicines has been developed in the form of Ayurvedic and Unani formulations in Bangladesh[4]. Proper scientific evaluation of the pharmacological properties of these plants, used in different formulations, would carry enormous potential and promise for the 21st century.

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and become a prime factor of morbidity and mortality in immune compromised patients in developing countries and many infectious microorganisms are resistant to synthetic drugs and hence an alternative therapy is very much needed [5]. Moreover, it is presumed that the broad spectrum effectiveness of plant species may provide a suitable basis for new antimicrobial therapies [6].

Cancer, the second leading cause of death worldwide next to cardiovascular diseases, is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, local tissue invasion, and distant metastases [7]. Multidisciplinary scientific investigations are making best efforts to combat this disease, but the sure-short, perfect cure is yet to be brought into world medicine. Plants have a long history of use in the treatment of cancer.

Over 60% of currently used anti-cancer agents are derived in one-way or another from natural sources, including plants, marine organisms and microorganisms [8].

Pain is defined as neuralgia, an unpleasant sensory experience associated with tissue damage, Such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause, or persist long after the precipitating injury has healed[9]. There are many herbs that are very useful and effective for pain relief. Many are safe for everyone but some should be avoided during pregnancy or while nursing [10].

Peltophorum pterocarpum (Copperpod, Golden Flamboyant, Yellow Flamboyant, Yellow Flame Tree, Yellow Poinciana and Radhachura in Bangla) is a family of Fabaceae, native to tropical southeastern Asia and a popularly ornamental tree grown around the world. Upon literature evaluation it was found that the plant is traditionally used in the treatment of unhealthy skin, ringworm, constipation, insomnia and stomatis [11]. The phytochemical investigation on *Peltophorum pterocarpum* leaves resulted in the isolation of eight flavonoids among which a unique flavone was able to inhibit acetylcholinesterase as well as possesses cardiotonic activity [12].

A mixture of steroidal glycosides (campesterol-3- 0- β- D- glucopyranoside, stigmasterol-3- 0- β- D- glucopyranoside and β- sitosterol- 3- 0- β- glucopyranoside) was isolated from the yellow fragrant flowers of *Peltophorum pterocarpum*. Bergenin, an isocoumarin was also found to be present in the flowers. The leaves, bark and wood of plant contain tannins [13].

Literature reviews indicated that no studies combining the analgesic, cytotoxicity as well as antibacterial of the flowers of *Peltophorum pterocarpum* have so far been undertaken. Taking this in view and as a part of our ongoing research [14, 15] on Bangladeshi medicinal plants, the present study aimed to evaluate the in vivo cytotoxicity of flower extract of *Peltophorum pterocarpum* along with their analgesic and antibacterial activities.

MATERIALS AND METHODS:

Plant materials: Flowers of *Peltophorum pterocarpum* were collected from the adjoining area of Rajshahi University Campus, Bangladesh during the month of April 2009 and were identified by Taxonomist, Department of Botany and University of Rajshahi, Bangladesh where a voucher specimen (Voucher No # 27) has been deposited for future references.

Preparation of extracts: The flowers of *P. pterocarpum* were dried in an oven at 37^{o}C and then pulverized into course powder with a mechanical grinder, passing through sieve #40, and stored separately in an air tight container. The dried powdered materials (1.0 kg) each, extracted three times by sonication for 30 min with MeOH: EtOAc (1:9) mixture (1000ml) and then filtered. The total filtrate of both was concentrated to dryness, *in vacuo* at 40^{o}C to render the crude extracts 120g.

Screening of *in vivo* cytotoxic activity:

Animal: Albino mice (25-30g) of both sexes were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum.* The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science & Technology, Dhaka, Bangladesh.

Transplantation of tumor: Ehrlich ascites carcinoma (EAC) cells were obtained from Indian Institute of Chemical Biology (IICB), Calcutta, India. The EAC cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation of 2×10^6 cells per mouse after every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7-8 of tumor bearing) of the tumor cells. Each animal received 0.1 ml of tumor cell suspension containing 2×10^6 tumor cells intraperitoneally.

Treatment schedule: 30 Swiss albino mice were divided into six groups (n = 6) and given food and water *ad libitum*. All the animals in each groups except

Group-I received EAC cells (2×10^6 cells/mouse i.p.). This was taken as day '0'. Group-I served as normal saline control (5 ml/kg i.p.) and Group-II served as EAC control. 24-h after EAC transplantation, Group-III received crude extract of *P. pterocarpum* at a dose of 50 mg/kg i.p. for nine consecutive days, respectively. Group-IV received reference drug Bleomycin (0.3 mg/kg i.p) for nine consecutive days [16]. Twenty-four hours of last dose and 18 h of fasting, 6 animals of each group were sacrificed by cervical dislocation to measure cytotoxic activity.

Analgesic activity:

Acetic acid-induced writhing test: 20 Swiss albino mice were divided into six groups (n = 5) and given food and water *ad libitum*. The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Test samples (Group III and IV) and vehicle (Group I) were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitoneally (Group II) 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min [17].

Antibacterial assay: Sterile 6.0 mm diameter blank discs (BBL, Cocksville, USA) were impregnated with test substances at the dose of 200 µg/disc. This disc, along with standard discs (Ciprofloxacin, Oxoid Ltd, UK) and control discs were placed in petri dishes containing a suitable agar medium seeded with the test organisms using sterile transfer loop and kept at 4°C to facilitate maximum diffusion. The plates then kept in an incubator (37°C) to allow the growth of the bacteria. The antibacterial activities of the test agents were determined by measuring the diameter of the zone of inhibition in terms of millimeter.

Antimicrobial activity was tested against *Bacillus anthracis***,** *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri***,** and *Shigella boydii* were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) [18].

Determination of Relative Percentage Inhibition: The relative percentage inhibition with respect to positive control was calculated by using the following formula [19]. Relative percentage inhibition of the test extract = [{100 x (a - b)}/(c - b)].

Where, a: total area of inhibition of the test extract; b: total area of inhibition of the solvent; c: total area of inhibition of the standard drug.

The total area of the inhibition was calculated by using area = πr^2; where, r = radius of the zone of inhibition.

Statistical analysis: All values were expressed as the mean ± standard error of the mean (SEM) of three replicate experiments and were analyzed using the GraphPad program (GraphPad, San Diego, CA, USA). The analysis was performed by using student's t test. $p<0.001$ were considered to be statistically significant.

RESULT AND CONCLUSION: *In vivo* cytotoxicity of extract against EAC tumor bearing mice was assessed by the parameter % of cell growth inhibition. Flower extract showed moderate cell growth inhibition and was found to be 30% (**Figure 1**). A number of scientific reports indicate certain terpenoids, steroids and phenolic compounds such as tannins, coumarins and flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis [20].

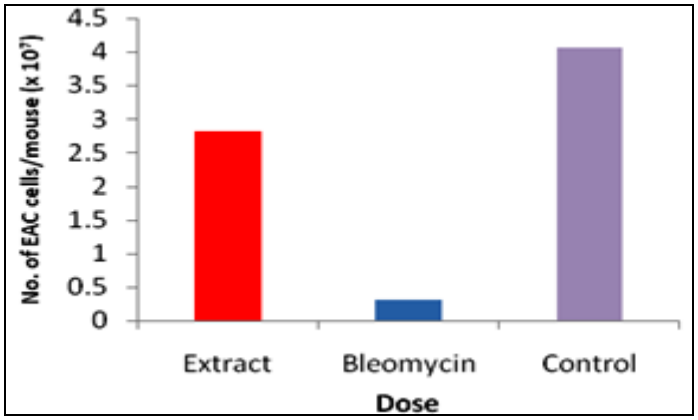

FIG. 1: *IN VIVO* CYTOTOXIC ACTIVITY OF FLOWER EXTRACT OF *P. PTEROCARPUM* ON EHRLICH ASCITES CARCINOMA (EAC) CELLS

Furthermore, flavonoids such as quercetin, kaemferol and their glycosides have been shown to possess antimutagenic and antiproliferative effect in various cancer cell line [21].

The *in vivo* cytotoxicity activities of *P. pterocarpum* are probably due to the presence of tannin, terpenoids, steroids as well as other bioactive compound. The extract effectively reduced the number of abdominal muscle contractions induced by 0.7% acetic acid solution in a dose dependent manner (**Table 1**). Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid [22].

TABLE 1: EFFECT *OF P. PTEROCARPUM* EXTRACT (FLOWER) ON ACETIC ACID INDUCED WRITHING IN MICE

Group	Dose in mg/kg	No. of writhing	% of Protection
Group-I (Control)	1% Tween 80 in water	26.33 ± 0.55	--
Group-II (Diclofenac Na)	Indomethacin	10.83 ± 1.22**	58.8
Group-III (Extract)	200	21.0 ± 1.69	20.24
Group-IV (Extract)	400	17.02 ± 0.76**	34.67

Values are Mean ± SEM (n=5); ** $p < 0.05$ by student's t test for values between the extract and the vehicle treated group

The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics the response is thought to be mediated by peritoneal mast cells [23], acid sensing ion channels [24] and the prostaglandin pathways [25]. **Table 2** expresses the antibacterial activity (zone of inhibitions) of the flowers of the *P. pterocarpum*. Flower extract at a dose of 200 µg/disc showed prominent activity against all the tested bacteria except *Staphylococcus aureus* where the zone of inhibition range was found to be 12 ± 0.32 to 18 ± 0.11 mm. The highest zone of inhibition was found against *Escherichia coli* and *Bacillus subtilis* (zone of inhibition of both 18 ± 0.02 and 18 ± 0.11 mm, respectively), followed by *Bacillus megatherium and Shigella boydii* (zone of inhibition of both 15 and 17 mm, respectively) whereas the moderate activity was shown against *Pseudomonas aeruginosa, Shigella flexneri, Bacillus cereus* and *Bacillus anthracis.*

TABLE 2:*IN VITRO* ANTIMICROBIAL ACTIVITY OF *PELTOPHORUM PTEROCARPUM* FLOWER EXTRACT

Bacterial strain	Diameter of zone of inhibition(mm)	
	[b]Std. (30µg/disc)	[a]Flower extract (200µg/disc)
Gram positive		
Staphylococcus aureus	27 ± 0.12	NA
Bacillus cereus	24 ± 0.10	14 ± 0.12 (34.02)
Bacillus megatherium	22 ± 0.02	15 ± 0.32 (46.48)
Bacillus subtilis	25 ± 0.22	18 ± 0.02 (51.84)
Bacillus anthracis	23 ± 0.02	14 ± 0.16 (37.05)
Gram negative		
Pseudomonas aeruginosa	25 ± 0.04	12± 0.32 (23.04)
Shigella flexneri	22 ± 0.13	13 ± 0.12 (34.91)
Shigella boydii	24 ± 0.17	17 ± 0.02 (50.17)
Escherichia coli	26 ± 0.10	18 ± 0.11 (47.92)

[a] Values of the observed diameter zone of inhibition (mm) excluding cap diameter. Incubation conditions for bacteria – 24 hours at 37°C. Assay was performed in triplicate and results are the mean of three values ± Standard Deviation. [b] Reference standard; Kanamycin. NA-Zone of inhibition < 5 mm consider as no activity. Parenthesis indicate the relative percentage of inhibition

Flower extract of *P. pterocarpum* showed the maximum relative percentage inhibition against *Bacillus subtilis* (51.84%) followed by *Shigella boydii* (50.17%) and *E. coli* (47.92%) whereas no relative percentage inhibition activity was found against *Staphylococcus aureus* (Table 2). Antimicrobial activities of tannins [26], flavonoids [27], saponins [28], terpenoids [29], and alkaloids [30] have been documented. Previous Phytochemical studies of this plant revealed that tannins, flavonoids, as well as steroidal glycoside are the main chemical constituents of plant. So, the highest antimicrobial activity showed by the extract of *P. pterocarpum* may be due to presence of such type of phytoconstituent.

CONCLUSION: The results of the present study indicate that *P. pterocarpum* exhibits interesting analgesic, cytotoxic as well as antimicrobial properties. These results of the investigation do not reveal that which chemical compound is responsible for aforementioned

activity. Now our next aim is to investigate the isolation and structure determination of the lead compound liable for aforementioned activity from this plant and work is going on in this respect in our laboratory.

REFERENCES:

1. Cragg MG, Newman DJ: Natural product drug discovery in the next millennium. Pharmaceutical Biology 2001; 39 (Suppl): 8–17.
2. Cordell GA: Pharmacognosy-new roots for an old science. In: Atta-ur-Rahman, Basha F.Z., eds. Studies in Natural Products Chemistry, Vol. 13: Bioactive Natural Products (Part A), Elseiver 1993; 629–675.
3. Kirthikar KR, Basu BD: Indian Medicinal Plants. 2nd Edition. Dehra dun, India, 1993; 220.
4. Okeke IN, Laxminarayan R, Bhutta ZA: Antimicrobial Resistance in developing countries. Part 1: recent trends and current status. Lancet Infect Disease 2005; 5: 481-493.
5. Kaushik P: Haridra (Turmeric): antibacterial potentials, Chowkhamba Sanskrit Series office, K 37/99, Gopal Mandir Lane, Varanasi, 2003; 16.
6. Dashora N, Sodde V, Bhagat J, Prabhu KS, Lobo R: Antitumor activity of Dendrophthoe falcate against Ehrlich Ascites Carcinoma in swiss albino mice. Pharmacutical Crops, 2010; 2: 1-7.
7. Newman DJ, Cragg GM, Snader KM: Natural products as sources of new drugs over the period 1981-2002. Journal of Natural Products 2003; 66: 1022-1037.
8. Rang HP, Dale MM, Ritter JM, Flower RJ: Rang and Dale's Pharmacology. Churchill Livingstone, International Edition, USA, 2003; 561.
9. Gallin JI: Fundamental Immunology. Raven Press, New York, 1989; 721.
10. http://en.wikipedia.org/wiki/*Peltophorum pterocarpum*
11. www.botanical.com. Medicinal herbs of Chattisgarh, India having less known traditional uses 64, Peela Gulmohar. (*Peltophorum pterocarpum*, family- cesalpiniaceae) Oudhia Pankaj.
12. Bhemachari J, Ashok K, Suresh DK, Gupta VRM, Narsimhachar J: Effect of *Arq Gauzaban* a Unanipathy product on the isolated Frog Heart. Acta Pharmaceutica Turica 2005; 47: 159 – 164.
13. www.google.co.in *Peltophorum pterocarpum* (Dc) Hayne, Houerou.
14. Rahman MH, Alam MB, Chowdhury NS, Jha MK, Hasan M, Khan MM, Rahman MS, Haque ME: Antioxidant, analgesic and toxic potentiality of *Stephania japonica* (Thunb.) Miers. Leaf. International Journal of Pharmacology 2011; 7(2): 257-262.
15. Alam MB., Chowdhury NS, Mazumder MEH, Haque ME: Antimicrobial and toxicity study of different fractions of *Dillenia indica* linn. bark extract. International Journal of Pharmaceutical Science and Research 2011; 2(4): 860-866.
16. Mazumder UK, Gupta M, Maity M, Mukherjee M: Antitumor activity of *Gyrophila spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. Indian Journal of Experimental Biology 1997; 35: 473-477.
17. Ahmed F, Hossain MH, Rahman AA, Shahid IZ: Antinociceptive and sedative effects of the bark of *cerbera odollam* Gaertn. Oriental Pharmacy and Experimental Medicine 2006; 6: 344-348.
18. Olurinola PF: A laboratory manual of pharmaceutical microbiology, Printed by National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria, 1996: 69.
19. Ajay KK, Lokanatha RMK, Umesha KB: Evaluation of antibacterial activity of 3, 5-dicyano-4,6-diaryl-4-ethoxycarbonyl-piperid-2-ones. Journal of Pharmaceutical and Biomedical Analysis 2002; 27: 837-840.
20. Blois MS: Antioxidant determination by the use of a stable free radical. Nature 2002; 26: 1199-1200.
21. Fotsis T, Pepper MS, Aktas E, Breit S, Rasku S, Adlercreutz H: Flavonoid, dietary-derived inhibitors of cell proliferation and *in vivo* angiogenesis. Cancer Research 1997; 57: 2916-2921.
22. Ahmed F, Selim MST, Das AK, Chowdhuri MSK: Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Pharmazie 2004; 59: 329-333.
23. Ronaldo AR, Mariana LV, Sara MT, Adriana BPP, Steve P, Ferreira SH, Fernando QC: Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. European Journal of Pharmacology 2000; 387**:** 111-118.
24. Voilley N: Acid-Sensing Ion Channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti-Inflammatory Drugs (NSAIDs). Current Drug Targets- Inflammation and Allergy 2004; 3: 71-79.
25. Hossain MM, Ali MS, Saha A, Alimuzzaman M: Antinociceptive activity of whole plant extracts of *Paederia foetida*. Dhaka University Journal of Pharmaceutical Science 2006; 5: 67-69.
26. Doss A, Mubarack HM, Dhanabalam R: Pharmacological importance of *Solanum trilobatum*. Indian Journal of Science and Technology 2009; 2(2): 41-43.
27. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A: Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. Journal of Applied Microbiology 2007; 103(6): 2056-2064.
28. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bially Z, Jurzysta M: Antimicrobial activity of saponins from *Medicago sp*.: structure activity relationship. Phytotherapy Research 2006; 20(6): 454-457.
29. Fnato K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, Hirari Y: Antimicrobial activity of hydrolysable tannins derived from medicinal plants against *Helicobacterp ylori.* Microbiology and Immunology 2004; 48(4): 251-261.
30. Navarro V, Delgado G: Two antimicrobial alkaloids from *Bocconia arborea*. Journal of Ethnopharmacology 1999; 66(2): 223-6.

****