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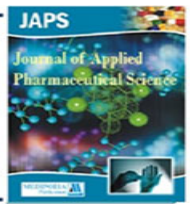

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Antinociceptive activity of the methanol extracts of leaves of *Eugenia fruticosa* (Roxb.) and *Glycosmis pentaphylla* (Retz.) in Swiss albino mice

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ABSTRACT

The present study was designed to evaluate analgesic potential of the methanolic extracts of the leaves of the both *Glycosmis pentaphylla* (Rutaceae) and *Eugenia fruticosa* (Myrtaceae). The analgesic activity was evaluated using the acetic acid (0.7% i.p.)-induced writhing inhibition method in swiss albino mice. The methanolic extract of leaves of *Glycosmis pentaphylla*, at the dose of 200 and 400 mg/kg body weight significantly (p<0.001) reduced the number of writhes with 31.97% and 44.21% of inhibition, respectively compared to control group. The methanolic extracts of leaves of *Eugenia fruticosa* significantly and dose dependently reduced the pain threshold (p<0.001) with 51.02 and 72.1% of writhing inhibition when compared to the control group at the dose of 200 and 400 mg/kg body weight, respectively which were comparable to that of the standard drug Diclofenac Na (65.31% inhibition of writhing, p<0.001). The results of the study demonstrated the potential antinociceptive activity of the methanolic extracts of the leaves of *Glycosmis pentaphylla* and *Eugenia fruticosa* which validated the traditional uses of the both plants in painful diseases and further investigations to elucidate the mechanism of action are required.

Keywords: *Glycosmis pentaphylla; Eugenia fruticosa*, Methanolic extract, Writhing, Acetic acid, Antinociceptive activity.

INTRODUCTION

Currently available conventional analgesic agents such as opiates and NSAIDs are not useful in all cases of painful conditions due to their adverse effects and thus new compounds with improved pain management capacity and fewer side effects are being sought with urgency (Jayaprakash, 2000). On the other hand, the long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine (Pattari *et al.*, 2010) . Besides, plant based treatment is more available and cost effective than the synthetic one. Another reason for increasing popularity of medicinal plants is its better compatibility and less side effects (Karim *et al.*, 2011). Most of the people living in developing countries still depend on plant-based traditional medicine for their primary health care. So search for plant based analgesic agents will be beneficiary for the management of pain and inflammatory conditions.

Glycosmis pentaphylla (Family: Rutaceae), an evergreen shrub or small tree is commonly called as orange berry and found from Bangladesh, India and Sri Lanka eastward to Myanmar, Thailand, southern China and Indo-China, possibly the Philippines, Peninsular Malaysia, Sumatra and Java and also cultivated elsewhere. *Glycosmis pentaphylla* (Rutaceae) are used traditionally for the treatment of boils, chest pain, hook worm infestation, ureterolithiasis (Uddin, 2006)**.** Juice of leaves is used in fever and liver complaints and as a vermifuge, leaves are considered good antidote for eczema and other skin troubles and applied in the form of paste. A decoction of roots is given for facial inflammations (Chopra *et al.,* 1969). Roots pounded and mixed with sugar are given in low fever and wood is used in snake bite (Chopra *et al.,* 1956). It is reported to contain arborinine (Quader *et al.,* 1999), glycozolicine, 3-formyl carbazole, glycosinine (Jash *et al.,* 1992), mupamine (Kamaruzzman and Chakraborty 1989), varbazole, 3-methyl carbazole (Chowdhury *et al.,* 1987), glycolone (Bhattacharyya and Chowdhury, 1985), glycozolidol (Bhattacharyya *et al.,* 1985), glycozolinine (Mukherjee *et al.,* 1983), glycophymoline (Sarka and Chakraborty 1979), glycophymine, glycomide (Sarka and Chakraborty, 1977), glycozoline (Chakraborty, 1969), noracronycine, des-N-methylacrocynine and des-N-methylnoracronycine (Govindachari *et al.,* 1966). *Eugenia fruticosa* (Myrtaceae), a small to medium sized evergreen tree is well known as bon jam, puti jam, khudi jam and traditionally has been used for the treatment of anemia, blood dysentery, dysentery (Uddin, 2006).

Though both the plants have potential traditional uses for the treatment of various diseases, there is no scientific study to validate their folkloric uses. Therefore, the present study was designed to evaluate the analgesic activity of the methanolic extracts of the leaves of the *Glycosmis pentaphylla* and *Eugenia fruticosa* by acetic acid induced writhing method in swiss albino mice.

MATERIALS AND METHODS

Drugs and chemicals

Acetic acid was obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Infusion Ltd., and Diclofenac Na was obtained from Square Pharmaceuticals Ltd., Bangladesh. All other reagents and chemicals used were of analytical grade.

Plant materials

For this present investigation, leaves of *Glycosmis pentaphylla* and *Eugenia fruticosa* were collected from Mirpur, Dhaka, Bangladesh in June, 2009 and were identified by the taxonomist of Bangladesh National Herbarium, MIrpur, Dhaka, where a voucher specimen has been deposited for future reference. The accession number of the plant *Eugenia fruticosa* and *Glycosmis pentaphylla* is DACB 34923 and DACB 34924, respectively. The collected plant parts were sun dried for one week and pulverized into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until extraction commenced.

Preparation of the extracts

About 150 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 200 ml of 95% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (methanol extract) obtained was evaporated using rotary evaporator. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of methanol. The extract was transferred to a closed container for further use and protection.

Experimental animals

Swiss albino mice of either sex weighing about 20-25 gm were used for the experiment. The mice were purchased from The Animal Research Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR,B). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hours light/dark cycle) for one week for acclimatization after their purchase and fed ICDDR,B formulated rodent food and water *ad libitum.* The design and performance of the study involving mice have been approved by the Ethical Review Committee for Animal Research, Ease West University, Mohakhali, Dhaka through the submission of a research protocol before the experiment.

Experimental procedures

Acetic acid induced writhing method

The analgesic activity of the samples was studied using acetic acid-induced writhing inhibition method in mice according to the method described by Koster *et al.* (Koster, 1969). Thirty swiss albino mice were divided into six groups of five mice each. Test samples of methanolic crude extract of *Glycosmis pentaphylla* and *Eugenia fruticosa* (200 and 400 mg/kg body weight for each sample respectively), vehicle (1% tween 80 in normal saline, 5 ml/kg body weight) and Diclofenac Na (50 mg/kg) were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid (0.1 ml/10gm). The forty minutes interval between the oral administration of test materials and intra-peritoneal administration of acetic acid was given to assure proper absorption of the administered samples. Five minutes after the administration of acetic acid, the numbers of squirms or writhes characterized by contraction of the abdominal musculature and extension of hind limbs was counted for each mouse over a period of 20 min. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was

compared to that of a control group while Diclofenac Na (50 mg/kg) was used as a reference substance (positive control). Percentage inhibition of writhing compared to control group was taken as an index of analgesia and was calculated using the following formula:

Inhibition (%) = [(Wc-W_t) /Wc X 100]

Where W_c is the average number of writhing reflex in the control group and Wt is the average number of writhing in the test groups.

Statistical Analysis

Data were expressed as mean ± SEM (Standard error mean) and were analyzed by One-way Analysis of Variance (ANOVA) followed by Dunnett's *t* test. Statistical significance was considered at $p<0.01$. The statistical analysis was carried out using the SPSS program (version 17.0).

RESULTS

Acetic acid-induced writhing response in mice

The crude methanolic extract of *Glycosmis pentaphylla* significantly ($p<0.001$) reduced the number of writhes with 31.97% and 44.21% inhibition compared to control group at the dose of 200 and 400 mg/kg body weight, respectively. On the other hand, the methanolic extract of *Eugenia fruticosa* significantly and dose dependently reduced the number of writhes ($p<0.001$) with 51.02 and 72.1% of inhibition when compared to the control group at the dose of 200 and 400 mg/kg body weight, respectively (**Table1**) which were almost similar to that of the standard drug Diclofenac Na (65.31% inhibition, $p<0.001$).

Table. 1: Effect of methanolic extracts of leaves of *Glycosmis pentaphylla* (GPL) *and Eugenia fruticosa* (EFL) on acetic acid induced writhing response in mice.

Treatment	Dose (mg/kg)	Writhing[a]	% inhibition
Control	10 mL	29.4±1.21	
Diclofenac Na	50	10.2±0.86*	65.31
EFL	200	14.4±1.56*	51.02
EFL	400	8.2±0.66*	72.1
GPL	200	20.0±1.58*	31.97
GPL	400	16.4±1.53*	44.21

[a] Values represent mean ± SEM (n=5). One -way ANOVA followed by Dunnett's *t* test, *$p<0.001$ compared to control.

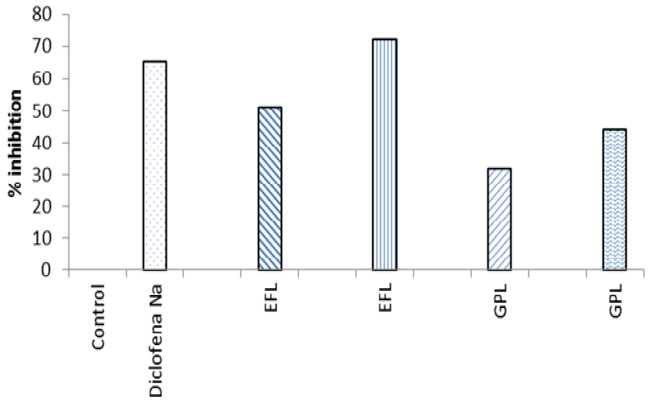

Fig. 1: Comparison of the % of inhibition of writhing effect of the methanolic extracts of leaves of *Glycosmis pentaphylla* (GPL) *and Eugenia fruticosa* (EFL).

DISCUSSIONS

The acetic acid induced writhing method is widely used to evaluate peripherally active analgesics. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting in the release of free arachidonic acid from tissue phospholipid via cyclooxygenase (COX), and prostaglandin biosynthesis (Duarte *et al.*, 1988; Ronaldo *et al.*, 2000). The released prostaglandins, mainly prostacyclin (PGI_2) and to lesser extent PGE_2 and $PGF_{2\alpha}$ have been held responsible for pain sensation (Derardt *et al.*, 1980).The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria *et al.*, 2008). So, the observed analgesic activity of the crude methanolic extracts of leaves of *Glycosmis pentaphylla* and *Eugenia fruticosa* might be due to their possible interference in the biosynthesis, release or action of prostaglandins and some other autacoids.

CONCLUSION

On the basis of results obtained from the present study, it can be concluded that the methanolic extracts of leaves of *Glycosmis pentaphylla* and *Eugenia fruticosa* plant possess potential analgesic activities and thus validated the traditional use of the plant in painful disorders. Present work is a preliminary effort which will require further detailed investigation including characterization of active compounds and investigation of possible mechanism of action of analgesic activity.

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