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Analgesic, cytotoxic and antioxidant activities of *Trewia polycarpa* bark

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SUMMARY

The crude ethanol extract of the stem bark of *Trewia polycarpa* (Family: Euphorbiaceae) was subjected to acetic acid induced writhing inhibition, Brine Shrimp lethality bioassay and 1, 1-diphenyl-2-picryl hydrazyl free radical scavenging assay for screening of analgesic, cytotoxic and antioxidant activity respectively. The extract produced significant ($P < 0.001$) writhing inhibition in acetic acid induced writhing in mice at the dose of 125, 250 and 500 mg/kg body weight respectively, which were comparable to the standard drug diclofenac sodium. The extract showed significant lethality to Brine Shrimp and the LC_{50} value was 8 µg/ml. The extract showed prominent free radical scavenging activity (IC_{50} about ~10 µg/ml) compare to standard drug ascorbic acid (IC_{50} about ~15 µg/ml). The results tend to suggest that the crude ethanol extract of the bark might possess analgesic, cytotoxic and antioxidant activities or active constituent(s) responsible for the activities.

Key words: *Trewia polycarpa;* Analgesic activity; Brine Shrimp; Cytotoxic activity; 1, 1-diphenyl-2-picryl hydrazyl

INTRODUCTION

Trewia polycarpa (Family: Euphorbiaceae) locally known as 'Pittali' or 'Medda', is almost a small to large deciduous, common plant and grows naturally in most places of Bangladesh (Gani, 2003). Bark contains a pentacyclic triterpene ketone, tetraxerone and beta-sitosterol (IJC, 1964). Decoction of leaves is used in swellings, healing of wounds, injuries and for the removal of bile, and phlegm. Decoction of shoots relieves flatulence and swellings on different organs or parts of the body. Decoction of roots is used in the treatment of flatulence, gout, rheumatism and as stomachic, alterative (Yusuf *et al.*, 1994). It has got different traditional uses in different localities of Bangladesh. There has been no scientific report on biological activity of bark part of this plant. We intended to investigate the analgesic, cytotoxic and antioxidant activities of the bark as a part of randomized screening.

MATERIALS AND METHODS

Plant material and extraction

The bark of the Trewia polycarpa were collected from Carmichael University College, Rangpur campus during the month of February, 2005 and was identified taxonomically by Forestry Discipline, Khulna university, Khulna. The specimen sample was preserved in the Phytochemistry Laboratory of Khulna University (No. PL-79). About 400 g of the bark was dried for 15 days without the direct contact of sunrays. The dried bark was finally

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ground and extracted by maceration over 20 days with 800 ml of 80% ethanol. The extract was filtered off. The solvent was evaporated under normal environment by an electric fan to get the dried extract(approx. yield value 9.7%). The crude extract was subjected to preliminary phytochemical screening for the detection of major chemical groups (Evans, 1989). The extract showed the presence of alkaloids, reducing sugar, gums, steroids, tannins. In each test 10% (w/v) solution of the extract in solvent was taken unless otherwise mentioned in individual test. This extract was used for pharmacological screening.

Animals

Male and female mice (*Swiss-webstar* strain, 20 - 25 g body weight) bred in the animal house of the Department of Pharmacy, Khulna University, were collected from animal resources branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) and used for the experiments. The animals were kept at animal house (Pharmacy discipline, Khulna University, Khulna) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2°C and 12 h light: dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water and maintained at natural day night cycle. The animals were divided in-groups with each group balanced for both sexes and body weights. We had ethical clearance for animal study from respective clearance committee. The investigation for cytotoxic property of the ethanolic extract was done on *Artemia salina* (Brine shrimp). The cyst of the shrimps was collected from the Oriental Fish processing industry, Khulna. One spoon of cyst were hatched for 48 hours in saline water, prepared by dissolving 20 g pure NaCl and 18 g normal edible NaCl into 1 L water. The cyst became nauplii.

Pharmacology

Acute toxicity test

Test animals (mice) were divided into different groups containing six animals in each. The groups received the bark extract orally at doses of 62.5, 125, 250, 500, 1,000, 2,000 and 4,000 mg/kg body weight whereas the control group received distilled water. General signs and symptoms of toxicity and mortality were recorded for 24 h (Lork, 1983).

Analgesic activity

Analgesic activity of the extract was tested using the model of acetic acid induced writhing in mice (Whitle 1964; Ahmed *et al.*, 2001). The experimental animals were randomly divided into five groups, each consisting of six animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 solution in water; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; group III, group IV and group V were test groups and were treated with the extract at the doses of 125, 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered

Table 1. Effect of *Trewia polycarpa* bark on acetic acid-induced writhings in mice

Treatment	Dose (mg / kg, p.o.) [a]	Writhings (n) [b]	Inhibition (%)
Control	1% Tween-80 solution in water	42.16 ± .215	-
Diclofenac sodium	25	8.35 ± .234*	80.19
T. polycarpa bark	125	24.75 ± .227*	41.29
T. polycarpa bark	250	15.33 ± .184*	63.64
T. polycarpa bark	500	9.83 ± .263*	76.68

[a]Administered 30 min before 0.9% acetic acid (60 mg/kg, i.p.). [b]Counted for 20 min after acetic acid injection; Values are mean ± S.E.M. (n = 6); n = Number of mice; *$P < 0.001$ vs. control, Student's *t*-test; i.p. = intraperitoneal; p.o. = per oral

Table 2. Result of Brine Shrimp lethality bioassay of *Trewa polycarpa* bark

Sample	Conc. of extract	Number of shrimps taken	Number of shrimps alive	Number of shrimps died	%Mortality
Ethanolic extract of *Trewa polycarpa* (stem bark)	2 μg/ml	10	10	00	0%
	4 μg/ml	10	10	10	0%
	6 μg/ml	10	06	04	40%
	8 μg/ml	10	05	05	50%
	10 μg/ml	10	04	06	60%
	20 μg/ml	10	04	06	60%
	50 μg/ml	10	02	08	80%
	100 μg/ml	10	01	09	90%
Chloramphenicol	200 μg/ml	10	0	10	100%

orally 30 min prior to acetic acid (0.7%) injection, then after interval of 15 min, the numbers of writhes (squirms) were counted for 5 min.

Cytotoxic activity

Solution of different concentrations was prepared with the extract by using dimethyl sulfoxide (DMSO) as solvent. Eight test tubes were used, in each test tube 10 shrimps were taken and solution of different concentrations applied on it. Finally volume of liquid was adjusted by saline water. The test tubes were kept for 24 h. For blank control, a test tube with saline water was kept for observation with 10 shrimps under the same condition with the test sample. For positive control, in another test tube 10 shrimps were taken with saline water. A known drug chloramphenicol as standard was introduced in the test tube with a concentration of 200 μg/ml (Table 2). The percent of mortality of the brine shrimp nauplii was calculated for every concentration to determine LC_{50} (lethal concentration).

Antioxidant activity

Free radical scavenging or antioxidant activity of the extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical.

Qualitative assay

A Suitably diluted stock solutions were spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 min and the color changes (yellow on purple background) were noted (Sadhu *et al.*, 2003).

Quantitative assay

Quantitative assay was performed on the basis of the modified method of Gupta *et al.* (2003). Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 mg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated (Fig. 2). The experiment was performed in duplicate and average absorbance was noted for each concentration. Ascorbic acid was used as positive control (Gupta *et al.*, 2003).

RESULTS

In the preliminary phytochemical screening, the bark extract showed the presence of alkaloids,

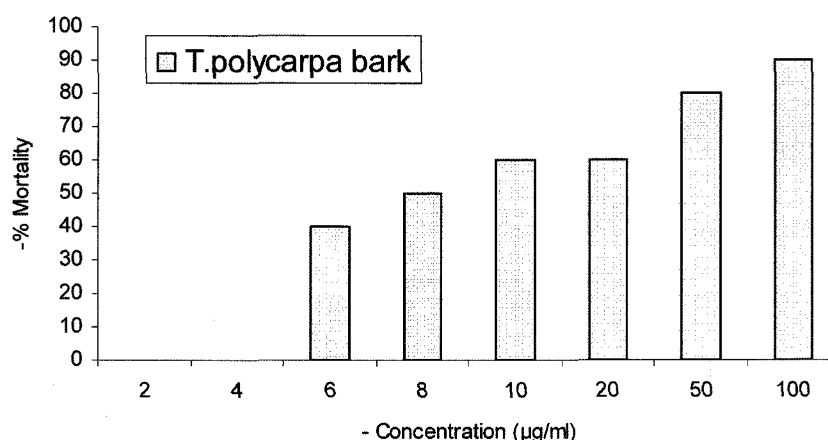

Fig. 1. Graphical representation of lethality bioassay.

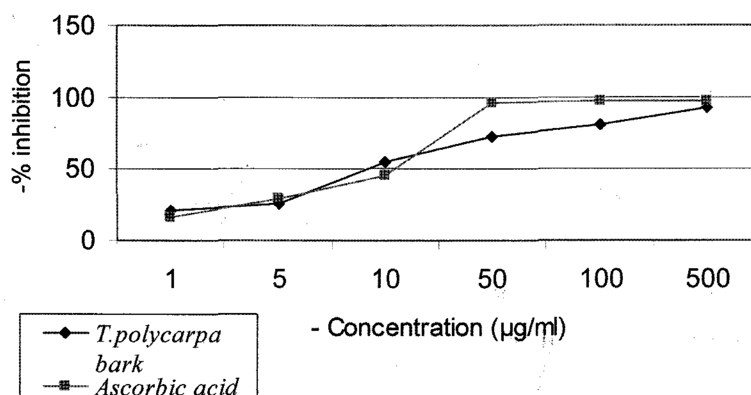

Fig. 2. Graphical representation of % inhibition of DPPH vs. Concentration.

reducing sugar, steroid, tannins and gums. In the acute toxicity test, the animal exhibited decreased mobility but no convulsions or loss of writhing reflex and at highest dose tested 4,000 mg/kg, no mortality was observed in the test animals. Analgesic activity was tested by using the acetic acid-induced writhing model in mice. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings (Taesotikul *et al.*, 2003). The extract produced about 41.29%, 63.64% and 76.68% writhing inhibition at the doses of 125, 250 and 500 mg/kg body weight respectively, which were comparable to standard drug diclofenac sodium where the inhibition was about 80.19% at the dose of 25 mg/kg (Table 1). The bark extract was found to show significant activity against the Brine Shrimp nauplii and LC_{50} was found at 8 µg/ml, which is very much lethal (Fig. 1). Antioxidant activity of the extract was performed on the basis of the modified method of Gupta *et al.* (2003). The extract showed prominent antioxidant activity (IC_{50} about ~10 µg/ml) against DPPH free radical which was significant compare to that of standard drug ascorbic acid (IC_{50} about ~15 µg/ml) (Table 3).

DISCUSSION

Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible

Table 3. Antioxidant activity of *Trewia polycarpa* bark

Sample	Concentration (μg/ml)	% inhibition	IC_{50} (μg/ml)
Et. extract of *Trewia polycarpa* (stem bark)	1	21	~10
	5	26	
	10	55	
	50	73	
	100	81	
	500	92	
Ascorbic acid	1	16	~15
	5	29	
	10	46	
	50	96	
	100	97	
	500	97	

Values are expressed as mean; Et.: Ethanolic

for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al.*, 1980). On the basis of the result of acetic acid induced writhing test, it can be concluded that the extract might possess an antinociceptive activity and the mode of action might involve a peripheral mechanism. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor etc. activity of the compounds (Meyer, 1982; Mclaughlin *et al.*, 1988). The rate of mortality was found to increase with the increase of concentrations which indicate a linear relationship between them. Further investigations are suggested using melanoma and carcinoma cell lines and to isolate the compounds responsible for this activity. Both *in vitro* and *in vivo* systems have been therefore developed to evaluate antioxidant activity of drugs which are useful in free radical-induced different types of cancer or carcinoma, diabetes, hypertension, tumor etc. The extract might possess constituents that confer H^+ to scavenge free radical DPPH. That is why with the increasing concentrations of both crude extracts and ascorbic acid it was found that % inhibition of free radical scavenging activity were also increased. Further studies as lipid peroxidation inhibition, xanthin oxidase inhibition, erythrocytic membrane stability and in vivo assays are essential to characterize them as biological antioxidants.

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