Evaluation of Anti-Ulcer Potential of the Leaf Extracts of *Platycladus orientalis* (L) Franco (Cupressaceae,) in Ethanol-induced ulcer in Rats

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Platycladus orientalis (L) Franco (PO) which is also known as Thuja has been used traditionally to treat various disease as diabetes, fever, epilepsy, pain, inflammation including peptic ulcer disease in Indian and chinese folk medicine, but its scientific efficacy in many disorder has not been validated. The present study was therefore carried out to evaluate the anti-ulcer activity of ethanol leaf extract of *P. orientalis* in rats. The effect of plant extracts on gastric ulcer in rats in ethanol-induced models was studied using dose (100, 200mg/kg). Omeprazole (50 mg/kg) were used as the standard drugs. On the ethanol-induced models, outcome measures were volume and pH of gastric fluid, total acidity, ulcer score, percent inhibition of ulcer score, ulcer index as well as percent inhibition of ulcer index. Data were analyzed using one-way analysis of variance and $P<0.05$ was considered as statistically significant. PO significantly ($P<0.001$) reduced gastric ulcer index by 62.12%, in ethanol-induced ulcer models by ethanolic extract at the 200 mg/kg dose, which is comparable to the standard drugs. Various extract of PO possesses both dose-dependent and time-dependent anti-ulcer effect in the animal model. The oral median lethal dose (LD50) is estimated to be higher than 2000 mg/kg for the crude alcoholic extract, and secondary metabolites such as terpenoids, flavonoids, alkaloid and saponins were present. The findings of this study confirmed that PO has anti-ulcer pharmacologic activity due to one or more of the secondary metabolites present in it. Therefore, this study validates its anti-ulcer use in Chinese and Indian folk medicine for use in gastric ulcer.

Keywords: Anti-ulcer activity, In vivo, *Platycladus orientalis*, Rat, Traditional.

Introduction:

Stress has become a very common problem in every household and it leads to many diseases. One among them is peptic ulcer [1] Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, representing a major burden for health care resources. [1, 2] Although potent anti-ulcer drugs are available, most of them produce several toxicities, thus emphasizing the need to search for new alternatives.[2, 3] As high as 80% of the world population depends on plant-derived medicines for the first line of primary health care,[3] reinforcing the theory that plant extracts can be good sources of new drugs. [4] Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue [5]. Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. [2, 5, 6]

Recently, there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most of the studies focus on newer and better drug therapy. These have been made possible largely by the availability of the proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog. [7, 8] This has been the rationale for the development of new antiulcer drugs, which includes herbal drugs. Indian Medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders. [8, 9, 10]

The genus *Platycladus* includes more than 60 species and belongs to the family Cupressaceae. ***Platycladus orientalis***, also called biota, morpankhi is a species of plant from the Cupressaceae family that was one of the original sources of thusa. Platycladus orientalis (L.) is a monoecious, multipurpose, evergreen plant which has been used anciently for its medicinal importance and association with long life and vitality in China and India. [11, 12] ***Platycladus orientalis*** contain various phyto-constituents such as alkaloids, glycosides, steroids, phenolic compounds, flavonoids and carotenoids. Thuja orientalis leaves contain rhodoxanthin, amentofl- avone, hinokiflavone, myricetin, carotene, xan- thophylls and ascorbic acid. The fruit and roots are strongly aromatic. Distillation of the dried leaves yields an essential oil. The composition of the oil is as: a new bicyclic sesquiterpene, l-borneol, bornyl acetate, α-thujone, camphor,and a new sesquirterpenenic alcohol. [12-18]

It is widely distributed in China and India used traditionally to treat **peptic ulcer** disease, cancer, Fever, Convulsion, Pain, Inflammation. Thuja is also occasionally used for treating diseases of skin, blood, gastrointestinal tract, kidney, brain, warty excrescences, spongy, hepato-protective and as antioxidant. Also used as Antimicrobial, Antioxidant, Anti-Inflammatory, Antidiabetic, anthelmintic etc. [19-30]

Methods and Materials:

Plant material collection and identification:

The fresh leaves of ***Platycladus orientalis*** (L) Franco. (Cupressaceae) were collected from local area **Alwar Rajasthan**, during the month of October-November. Taxonomic identification was established by ethno botanist. Two herbarium were prepared one was sent to Department of Botany for proper authentication and a voucher specimen was submitted at Institute's herbarium department for future reference no. SRU 128/11

Experimental animals:

Healthy adult Wistar albino rats of either sex were selected randomly for the study. The rats were obtained from the animal house of the Department of Pharmacology, Ponda Education Society's Rajaram and Tarabai Bandekar College of Pharmacy. (**CPCSEA Reg. No.- 1659/PO/a/CPCSEA**) Rats of 12–16 weeks, weighing 160–200 g, were used for the experiment. Each rat was housed in a plastic box cage under standard conditions at 20–25°C and was kept under 12/12 h light/dark cycle. The rats were allowed free access to balanced diet (standard pellet feed) and water ad libitum. The study was carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals and Organization of Economic Co-operation and

Development (OECD) guidelines. Approval from the Research Review Committee of the Department of Pharmacology was also obtained.

Ethical Approval:

The study was approved by the animal ethics of Ponda Education Society's Rajaram and Tarabai Bandekar College of Pharmacy and the procedures are followed the format given in form-B of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA).

The selected rats were divided into five groups, each group consisting of 6 animals for pharmacological screening.

Table 1: Grouping and dose of drug

Model of Stress	Drug and Doses
Ethanol induced Stress	
Group I	Control (Received D/W)
Group II	P. orientalis 100mg (i.p.) and ulcerogenic materials
Group III	P. orientalis 200mg (i.p.) and ulcerogenic materials
Group IV	Omeprazole 50mg/kg (oral) and ulcerogenic materials

Chemicals and Drugs:

Chemicals:

All chemicals and reagents used in this study like Glacial acetic acid, benzene, chloroform, ethanol, ferric chloride, mercuric chloride, potassium iodide, methanol, lead acetate, sulfuric acid, hydrochloric acid, sodium hydroxide, phenolphthalein for the purpose of Phytochemical investigation and extraction were of analytical grade and obtained from Merck Company, Germany and various other company.

Omeprazole: Obtained from Dr. Reddy's Laboratories Limited and used as standard drug. It was suspended in distilled water and administered orally 50mg/kg (oral) once a day (OD) for 7 days to rats by using feeding tube.

Preparation of plant extract:

The plant material was dried under shade at room temperature for about 5 days. The dried plant samples were coarsely powdered by mechanical grinder and sieved to give particle size 100 to 150 mm. The course powder of leaves was extracted with ethanol solvent in Soxhlet apparatus (Hot continuous percolation) at a temperature not exceeding 60 °C as plant contain mainly volatile oil. The extracts were concentrated under reduced pressure in a rotary evaporator to yield a crude semi-solid mass. It was then dried and used.

A portion of residue from each extract was subjected to phytochemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, sterols and tri- terpenoids in the leaves extracts. The

preliminary phyto- chemical screening was performed with the standard procedures and the nature of the phytoconstituents was identified. [15, 16, 18, 20]

Preliminary phytochemical and Physico-chemical screening:[31]

The extracts of *P. orientalis* leaves was tested for the presence of various phytoconstituents such as carbohydrate, alkaloids, glycoside, phenolic compound and saponins, flavonoids, fixed oils and fat test. Physicochemical values such as the foreign organic matter, moisture content, ash value as well as extractive value were determine as per the official methods (Anonymous,1996) as well as per WHO guidelines of quality control method for medicinal plant materials (WHO,1998; WHO,1992)

Acute toxicity test:

The main aim of acute oral toxicity study is to determine the therapeutic index. The acute oral toxicity was carried out as per the guidelines set by Organization for Economic Co- operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. [15]

A limit dose of 2000 mg/kg of extract was administered sequentially and animals were observed individually for behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation and urination), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and for morbidity or mortality, after dosing continuously for 2 hours, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days. [16, 18]

Ethanol-induced ulcer model: [10, 29, 30].

Absolute ethanol was administered by orogastric tube at a dose of 5 ml/kg. After 1 hour of ethanol administration the animals are sacrificed. The abdominal cavity was opened through a midline abdominal incision to expose stomach. The stomach dissected out, opened along the greater curvature and washed in normal saline. The stomachs were observed for the ulceration with the help of magnifying lens and studied its external, internal surface and ulcer index was evaluated according to the severity of ulcers.

Statistical analysis:

All values were reported as mean + S.E.M. The statistical significance of differences between groups was assessed using one-way ANOVA. A probability value of $p < 0.05$ was considered to be statistically signify

Results:

Phytochemical screening:

Preliminary phytochemical screening of *P. orientalis* confirmed the presence of different secondary metabolites, as shown in the Table 2.

Table 2 Preliminary phytochemical test of extract of *Platycladus orientalis*

S.No.	Phyto Constituents	Phytochemical tests	Results
1	Carbohydrate	Fehling's test	Positive
2	Alkaloids	Dragendroff's, Mayer's, Wagner's, Hager's test	Positive
3	Glycoside	Keller-Killani test With con. H_2So_4	Positive
4	Tannins	With $FeSO_4$, lead acetate test and ferric chloride	Negative
5	Saponins	Foam test	Positive
6	Flavonoids	With NaOH, with lead acetate test, H_2So_4	Positive
7	Fixed oils, fat test	Spot test	Positive
8	Volatile Oil	Sudan III, Tincture Alkane	Positive

Table: 3 Physicochemical parameter as Ash values, Loss on drying, Volatile oil content

Evaluation Parameter	Value
Total ash value	16.12 ± 0.54 % w/w
Water soluble ash value	4.54 ± 0.66 % w/w
Acid insoluble ash value	14.34 ± 0.76 % w/w
Sulphated ash value	13.58 ± 0.88 % w/w
Loss on drying	8.79 % w/w
Volatile oil	1.10 % w/w
Foreign matter	1.78% w/w

Acute toxicity test:

With the acute toxicity test at the limit test dose of 2000 mg/kg, all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. Neither mortality nor changes related to behavioral, autonomic, and physical profiles were observed within the first 24 hours and during the 14-day follow-up. From these results it is concluded that the extract is quite safe even at these higher doses and had no acute toxicity and the oral lethal dose (LD50) for the male and female rats was greater than 2 g/kg body weight.

Anti-ulcer activity evaluation:

Ethanol-induced gastric ulcer/oxidative stress:

The ulcer was induced by administering ethanol following the method by Dashputre and Naikwade.[19] All the animals were fasted for 48 hours before administration of ethanol. The gastric ulcers were induced in rats by administrating ethanol (50%) (5ml/kg) orally, after 60 minutes of various extract treatment. They were kept in specially constructed cages to prevent coprophagia during the experiment. Ulcer control group was orally administered with vehicle (carboxymethyl cellulose, CMC, 0.25% w/v, 5ml/kg). The reference group received

oral doses of 50 mg/kg Omeprazole in CMC (5 ml/kg) as positive controls. Experimental groups were orally administered with 100 and 200 mg/kg of different extract of *P. orientalis* leaf in CMC solution (5 ml/kg), respectively. One hour after this pre-treatment; all groups of rats were gavaged with absolute ethanol (5 ml/kg) in order to induce gastric ulcers (Hollander et al., 1985). The animals were anesthetized 1 hour later with anesthetic ether, and the stomach was incised along the greater curvature and ulceration was scored. [31-36]

The percentage of ulcer inhibition calculated by formula as described by Njar et al. [31]

$$\%\,\text{of Ulcer inhibition} = \frac{\text{Mean UI (control) - Mean UI (treated)}}{\text{Mean ulcer index of control}} \times 100$$

[UI= Ulcer index]

Evaluation of ulcers score: [32, 33]

0 - No pathology, 1 - A small ulcer (1-2mm), 2- Medium ulcer (3-4 mm), 4 – Large ulcer (5-6 mm), 8 – Large ulcer (> 6 mm)

Determination of pH: [34, 35]

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water, and pH of the solution was measured using pH meter

$$\textbf{Ulcer index (UI)} = \frac{\text{Total severity of score}}{\text{Number of animals}}$$

Table: 4 Effect of the different Extractives of *Platycladus oriantalis* on Ethanol Induced Gastric Ulcers in Rats

Treatment	Dose (mg/ kg b.w)	Ulcer Index	% Ulcer Inhibition
Control	10 ml/ kg	34.12 ± 0.16	-
Petroleum Ether extract	100	28.34 ± 0.35NS	3.88
	200	30.2 ± 0.39 NS	5.21
Chloroform extract	100	27.8 ± 0.32 NS	7.88
	200	28.6 ± 0.23*	8.76
Ethyl acetate extract	100	17.6 ± 0.45**	40.22
	200	15.8 ± 0.65**	45.32
Ethanol extract	100	13.5 ± 0.24**	56.16
	200	**10.9 ± 0.21****	**62.12**
Water extractives	100	16.8 ± 1.02**	42.24
	200	12.3 ± 0.4**	56.12
Omeprazole	**50**	**8.2 ± 0.4****	**72**

Notes: Each value represents the mean ± SEM for each group (n=6); aagainst nc; **P<0.01; **P<0.001.

Abbreviations: SEM, standard error of mean.

Data presented as Mean ± SD, * P < 0.05; ** P < 0.001; ns P > 0.05

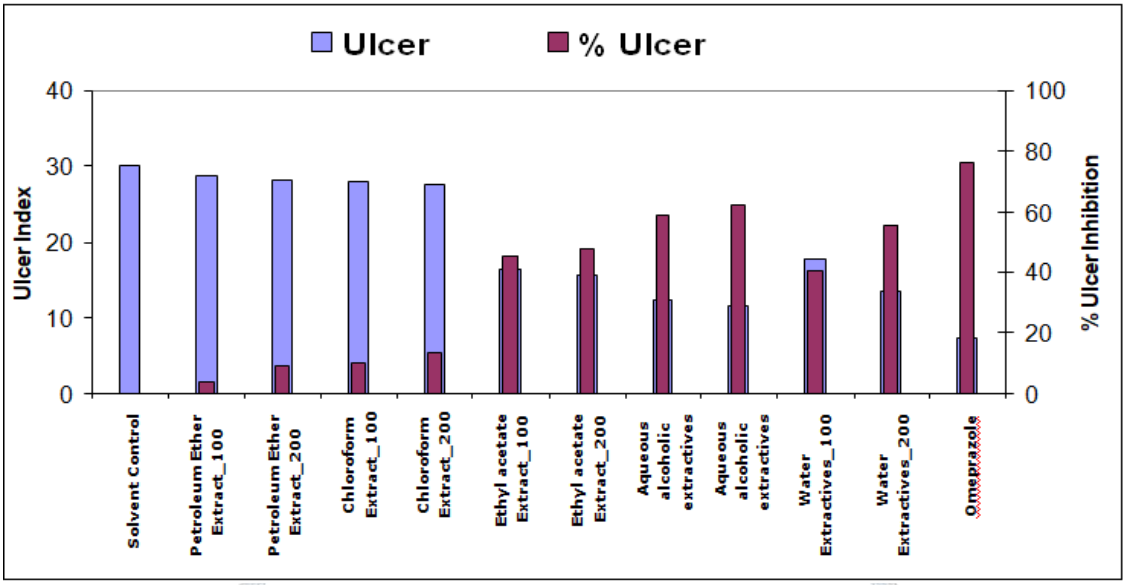

Figure: 1 Comparative Effect of *Platycladus oriantalis* Leaf Extractives on Ethanol Induced Gastric Ulcers in Rat

Gross appearance of gastric mucosa in rats:

On gross examination the gastric mucosa in ethanol administered group showed dilated blood vessels, haemorrhagic sites and large number of pin point ulcers of varying sizes with central clots, features of perforation in the stomach. The ulcer index was high in control group. Animal treated with extract at the dose of 100 and 200mg/kg showed few sign of mucosal injury and the percentage of damage were less compared to control group. Corresponding ulcer index also was reduced. These features were suggestive of anti ulcer activity of *Platycladus orientalis*. Animals treated with omeprazole maintained near normal pattern. The ulcer index was markedly reduced. Various Anti-secretary Parameters of ethanol extract shown in table 5

Table:5 Effect of EEPO on Anti-secretary Parameters in ethanol induce ulcer

Treatment	Dose (mg/ kg *b.w*)	Gastric Volume (ml/100g)	pH	Free Acidity (mEq/l/ 100g)	Total Acidity (m Eq/ l/ 100g)
Control	10ml/ kg	2.52±0.31	1.43±0.12	67.01±3.1	76.84±3.8
EEPO	100	1.54±0.24 NS	2.02±0.22**	48.32±0.22**	56.32±3.56**
EEPO	200	1.74±0.42*	2.82±0.44**	35.4 2±2.66**	40.42±2.46**
Omeprazole	50	1.68±0.07**	4.44±2.55**	24.13±3.5**	32.93±3.5**

Values are expressed in terms of mean ± S.E.M, ** p<0.01 Vs control - One way ANOVA followed by Dunnett's test.EEPO- *Ethanol Extract of Platycladus orientalis*

Discussion:

This study was carried out to evaluate the anti-ulcer effect of various leaf extract of PO on ethanol-induced gastric ulcer models. The 21.12% plant extraction yield is similar with the 24.65%and 26.12% reported in previous studies done in China and India, thus supporting that hydroalcoholic solvent possesses a good extracting potential. Ethanol releases more diverse phytochemicals such as tannins, saponins, terpenoids rhodoxanthin, amentofl- avone, hinokiflavone, myricetin, carotene, xan- thophylls, ascorbic acid, lectones, flavones, phenols, and polyphenols than other extraction solvents. [36, 37, 38] The acute toxicity study revealed that the plant extract was safe in rats at a limit dose of 2000 mg/kg and that the median lethal dose (LD50) of the extract is above 2000 mg/kg. In the preliminary phytochemical screening, the ethanol leaf extract of PO was positive for flavonoids, saponin, phenols, terpenoids, and alkaloids. These secondary metabolites are effective as antioxidant, antineoplastic, anti-ulcer, anti- inflammatory, anti epileptic and immune stimulating agents.[38-40] Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, inhibit Helicobacter pylori growth, act as free radical scavengers, and inhibit H+/K+-ATPase. [41-43] Saponins may activate mucous membrane protective factors, and tannins render the outermost layer of the mucosa less permeable, for instance, to chemical irritation.[41] In addition, terpenoids and alkaloid compounds are also reported to have potent activity against gastric ulcers.[44, 45]

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis. [43, 46-48]

In the present study, the ethanol-induced model was employed to confirm the gastric cytoprotective effect of the plant extract. Antiulcerogenic activity of *Platycladus orientalis* ethanol extract was seen at the dose 100mg/kg & 200mg/kg and was almost comparable to the standard drug Omeprazole. Various extract and standard drug-treated groups showed a significant reduction (at least $P<0.01$) in both the ulcer score and ulcer index. The extract effect increases with the dose and is comparable with that of the standard drug. This finding signifies that the extract possesses a gastroprotective effect, which is as good as the standard drug. The results of the present study showed that the PO leaf extract was capable of inhibiting gastric lesions formed by ethanol. Absolute alcohol would extensively damage the gastric mucosa leading to increased infiltration of neutrophils, which are a major source of inflammatory mediators. Therefore, suppression of neutrophil infiltration during inflammation was found to enhance gastric ulcer healing. [38, 39, 40] Various Anti-secretary Parameters of 200mg/kg extract was also comparable with standard drug. Ethanol extract of PO leaf extract has been shown to contain anti-inflammatory activity, [23, 49] and it is speculated that the gastroprotective effect exerted by this plant could be credited to its anti-inflammatory property. Compounds that act as antioxidants or activate

the redox system are important for restoring gastric tissue. [23,40] Therefore, strong ulcer healing effect of the extract in the ethanol-induced model might also be related to the antioxidant activity of the plant, which is well demonstrated in previous studies.[14] Flattening of the mucosal folds observed in the present study also suggests the gastroprotective effect of the extract. Different reports showed that the changes in the gastric motility may play a role in the development and prevention of experimental gastric lesions. Relaxation of circular muscles will increase the mucosal area to being exposed to necrotizing agents and reduce the volume of the gastric irritants on rugal crest, leading to protection of the gastric mucosa against damage. [50-52]

Conclusion:

The findings in this study confirm the absence of acute toxicity at the doses employed, and presence of anti-ulcer pharmacologic activity of various PO extracts. Its efficacy is comparable to the standard drugs. Anti-ulcer effects may be related to antisecretory as well as cytoprotective activities of one or more of the identified phytochemicals. Thus, the present work validates the use of PO for gastric ulcer in the Indain and Chinese folk medicine, and further studies shall focus on isolation of specific phytochemicals and elucidating mechanisms of action.

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Disclosure:

The authors report no conflicts of interest in this work.

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