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Antidiarrheal, antisecretory and intestinal smooth muscle relaxant effects of *Platanus orientalis* in mice

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Abstract: *Platanus orientalis* is traditionally used to treat diarrhea and spasm. However, studies are lacking on its mechanism of action in diarrhea and spasm. Pharmacological *in-vivo* activities were performed. *In-vitro* activities were carried out to explore the underlying mechanism(s) of action in isolated tissue preparations of mice jejunum and ileum. Crude extract of *Platanus orientalis*, loperamide and verapamil were used. The crude extract provided dose-dependent protection in castor oil diarrhea like verapamil and reduced the intestinal fluid accumulation and charcoal meal transit distance. *In-vitro* studies produced spasmolytic effect on the spontaneous (EC_{50} value=0.21mg/mL), high K^+ (EC_{50} value=0.37mg/mL) and carbachol (CCh)-induced contractions 5.35mg/mL (3.88-6.85) respectively. The quiescent ileum responded well to the high K^+ and carbachol (CCh)-induced contractions when tested against crude extract. It caused inhibition of the induced contraction with EC_{50} values of 0.20mg/mL (0.10-0.30) and 3.25mg/mL (2-4.5) respectively and showed potent effect against CCh-induced contractions. Calcium response curves produced a similar effect to verapamil. The crude extract of *Platanus orientalis* remained safe up to 5g/kg dose.

Keywords: *Platanus orientalis*, antidiarrhea, antisecretory, antispasmodic, Ca^{+2} antagonist, BALB/c mice

INTRODUCTION

Traditional medicines exist in different systems influenced by geographic and environmental conditions of that region, beliefs and experiences of people make the foundation of traditional medicines (Yimer *et al*., 2021). When knowledge progressively developed from darkness, the use of floras as a source for curative procedures and preventing ailments turn out to be a subject of interest (Liao *et al*., 2022). In technologically advanced countries the use of traditional medicines has steadily increased (Panossian *et al*., 2021).

In USA, the projected "herbal" sale in 1995 was $2.5 billion and in 2005 it was increased to $4.4 billion. From 2005 to 2007, 13 drugs resultant of natural products were allowed in US (Balogun *et al*., 2019). The goal of WHO strategy, 2014-2023, is to build up the starring role of traditional medicine, accentuating the significance of encouraging and including the consumption of remedial plants in the health maintenance system of its adherent states (Les *et al*., 2021).

Gastrointestinal secretions are principally controlled through cholinergic, histaminergic as well as peptidinergic pathways especially gastrin (Geibel, 2022). Disturbances in above mentioned pathways results in hypersecretions of gastric acid triggering peptic ulcer disease as well as gastroesophageal reflux disease which may be refractory leading to perforation and bleeding (Gros *et al*., 2021). Diarrhea is a shift in the normal bowl movements described by escalation of water contents, bulk and frequency of stools in comparison with the healthy individual routine (Colmier *et al*., 2021). 1.6 million people deceased from diarrheal disease globally in 2017 (Melese *et al*., 2019). Amongst South Asian countries, diarrhea-related deaths are highest in Pakistan (Ali *et al*., 2022). The prevalence of diarrhea in Pakistan was 19% in 2017-18, yet this percentage is one of the highest in the region (NIPS, 2018).

Platanus orientalis belongs to the family Platanaceae, worldwide recognized as 'plane tree family' in the major angiosperm group. This is a huge, woody plant, attaining a peak of 55-60 m has a spreading crown and is recognized for its long existence (Niknam *et al*., 2021). It is scattered throughout Europe, Turkey, Afghanistan, Iran, India and Pakistan. *Platanus orientalis* is recognized by following names; chinar or chinar (Urdu/Hindi), boonyi (Kashmir valley region), oriental plant (English) and dulb in Arabic (Haider *et al*., 2012).

Hakim Momen and Avicenna have mentioned the medicinal effectiveness of *Platanus orientalis* in their books for its anti-inflammatory and analgesic effects in knee pain and toothache (Haider *et al*., 2012; Ibrar *et al*., 2015). The leaves and flowers of this plant are traditionally used for treating hemorrhoids, ophthalmologic agent, diabetes cure, antipyretic, diaphoretic, in kidney stones, rheumatism, arthritis, burns, abscess treatment and wound healing activity (Nishanbaev *et al*., 2004; Bulut and Tuzlaci, 2013; Sargın *et al*., 2013). Roots of this plant are used as anti-venom in case of snake bite and as a haemostatic agent (Nishanbaev *et al*., 2004). Leaves and bark of *Platanus orientalis* are used for treating gut disorders (Priya and Sharma, 2014; Ajmal *et al*., 2012) and for discharging puss from wounds (Ibrar *et al*., 2015).

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This plant has been studied for different pharmacological activities including anti-inflammatory and anti-nociceptive activities. The polyphenolic and hydroalcoholic extracts of *Platanus orientalis* possess adequate anti-nociceptive activity (Hajhashemi *et al*., 2011). The ethanolic extract as well as fractions particularly the chloroform fraction exhibited major analgesic activity and histopathological study showed insignificant ulceration on comparison with Ibuprofen i.e., the standard drug (Haider *et al*., 2012).

MATERIALS AND METHODS

Drugs and Chemicals

Chemicals of analytical grade were got from sources specified: Atropine sulphate, acetylcholine chloride, histamine diphosphate salt, potassium chloride, charcoal meal, loperamide hydrochloride and verapamil hydrochloride were bought from Sigma Aldrich Chemicals Company, St. Louis, MO, USA.

The castor oil was bought from Karachi Chemical Industries, Karachi, Pakistan. For preparing the stock solutions for standard drugs distilled water was used and normal saline/distilled water was used for dilutions being freshly prepared before the experiment. Methanol was used for extraction. Chemicals of highest purity grade used were. Solvents used for solubilization had no consequences on experiments.

Animals

The experiments done met terms of Commission on Life Sciences, Institute of laboratory animal resources, National Research council (NRC, 1996). In the animal house of Pharmacy department, COMSATS University Islamabad, Abbottabad Campus. BALB/c male mice weighing 20-25 g, 4-6 months old, were housed and fed with diet, which is standard, set at 12 hours light-dark cycles at 23-25 ℃.

Collection of plant material

The leaves of *Platanus orientalis* were collected from the District Abbottabad, Khyber Pakhtunkhwa, Pakistan and given the voucher specimen number DB-GC (AGC)-0033.

Extraction of plant material

After collection process the leaves were cleansed and shade dried. Dried leaves were powdered and cold macerated with 70% methanol for 15 days with occasional shaking. The material was filtered using muslin cloth and filtration process completed through Whatmann filter paper of Grade 1. The filtration process was repeated thrice to get maximum number of constituents.

Filtrate was concentrated using rotary evaporator (Rotavapour Model HAHN VAPOR HS-2005S-N, Korea) being attached to chiller (RW-0525, Jiec Tech, Korea), electric aspirator (HS-3000, Korea) was used to reduce the pressure to -760mmHg and obtained 12.45% (w/w) crude extract (Niknam *et al*., 2021).

Pharmacological investigations

In-vivo studies

Acute toxicity test

Acute toxicity test was performed according to the method described earlier (Sosa *et al*., 2020). Dose up to 5g/kg was administered to BALB/c mice through oral route. Animals were observed for behavioral changes, breathing and mortality.

Effects of crude extract of Platanus orientalis on castor oil-induced diarrhea in mice

This activity was conducted as described earlier (Saqib *et al*., 2021). Divide BALB/c mice after keeping at fasting for 18 hours, into eight groups. One group received normal saline (10mL/kg, p.o.). Another received castor oil. Three groups received *Platanus orientalis* crude (100, 300 and 1000mg/kg, p.o.). Verapamil was given as a standard dose (1, 3 and 10mg/kg, p.o.). After one hour, 10mL/kg, p.o. castor oil was administered and observed for defecation. After four hours diarrheal dropping was noted on each blotting sheet of each cage. Percentage protection was calculated based on comparison of dry feces with wet feces.

Effects of crude extract of Platanus orientalis on castor oil-induced intestinal fluid accumulation in mice

This activity followed the method earlier conducted by (Rakulini *et al*., 2019). After overnight fasting, BALB/c mice were arranged into six groups. Normal saline (10mL/kg, p.o.), castor oil, crude extract of *Platanus orientalis* (100, 300 and 1000mg/kg, p.o.), loperamide (10mg/kg) were administered to different groups. After one hour castor oil (10mL/kg) was administered. Cervical dislocation was accomplished 30 minutes later, entire intestine was removed. Expressed the result as (*P*i/*P*m) x 1000.

Effects of crude extract Platanus orientalis on gastrointestinal transit time in mice

This activity followed the method conducted earlier by (Stamatopoulos *et al*., 2021). The mice after overnight fasting were allocated to five groups. Normal saline, crude extract of *Platanus orientalis* (100, 1000 and 3000mg/kg) and loperamide (1 mg/kg) were administered. Each animal was administered with charcoal meal one hour later orally and the mice were sacrificed, the charcoal transport ratio is obtained.

In-vitro studies

Effects of crude extract of Platanus orientalis on mice jejunum and ileum

This activity followed the method performed earlier on jejunum and ileum tissue of mice (Ali *et al*., 2020). Experimental mice were kept at overnight fasting, cervical dislocation being done to sacrifice the animal. 2-3 cm portions of ileum and jejunum being isolated out clearing off mesenteries. Introduced tyrode solution in tissue baths (10mL). The prepared tissues were mounted in the tissue

bath where preload was set equivalent to 1 g for jejunum tissue and 0.5-1g was adjusted for ileum tissue. Extract was verified against contractions induced by carbachol and high K^+ (80mM) in equilibrated tissues.

Determination of calcium channel blockade activity of crude extract of Platanus orientalis on mice jejunum and ileum

After stabilizing tissues, high K^+ (80mM) was given for constant contraction. Crude extract of *Platanus orientalis* was added cumulatively to obtain super imposed CRCs, tissue was pretreated with extract dose to evaluate calcium channel blocking activities (Mapesa *et al*., 2021).

STATISTICAL ANALYSIS

Statistical parameters used were student's *t*-test, $p<0.05$ (Graph-Pad version 8, San Diego, Ca, USA), mean± standard error means (SEM) and median effective concentrations with 95% confidence interval (CI).

RESULTS

In-vivo studies

Acute toxicity test

Crude extract of *Platanus orientalis* was safe up to 5g/kg with no lethality being observed. The breathing and general behavior of the animal was also observed normal.

Effects of crude extract of Platanus orientalis on castor oil-induced diarrhea in mice

Percent protection was found to be 22.36% (100mg/kg), 59.76% (300mg/kg) and 79.20% (1000mg/kg) significantly ($p<0.05$) with respect to the control group and groups treated with castor-oil shown in table 1.

Effects of crude extract of Platanus orientalis on castor oil-induced intestinal fluid accumulation in mice

The crude extract of *Platanus orientalis* resulted 143.3±3.81 at 100mg/kg, 123.5±9.29 at 300mg/kg and 95.30±1.35g at 1000mg/kg ($p<0.05$, 0.01 and 0.001). Loperamide treated group resulted 84.20±2.61g ($p<0.001$) at 10mg/kg shown in table 2.

Effects of crude extract of Platanus orientalis on charcoal meal gastro-intestinal transit time in mice

Results obtained were 67.21±9.70 at 100mg/kg, 50.66 ± 9.55 at 1000mg/kg and 43.92±10.97 at 3000mg/kg, whereas 61.33 ± 9.615 at 1 mg/kg loperamide, shown in table 3.

In-vitro studies

Effect of crude extract of Platanus orientalis on mice jejunum

It caused inhibition of spontaneous [EC_{50} = 0.21mg/mL (0.13-0.30)], high K^+ [EC_{50}=0.37(0.22-0.50)] and carbachol (CCh) induced contractions [EC_{50}= 5.35(3.88-6.80)] in isolated mice jejunum tissue preparation (fig 1. A-F and fig 2. A, B).

Crude extract of Platanus orientalis on mice ileum

Crude extract of *Platanus orientalis* resulted in the inhibition of high K^+ [EC_{50}=0.20mg/mL (0.10-0.30)] and carbachol (CCh) induced contractions [EC_{50}=3.25mg/mL (2.0-4.5)] in isolated mice ileum tissue preparation (fig 3. A-C and fig 4. A, B).

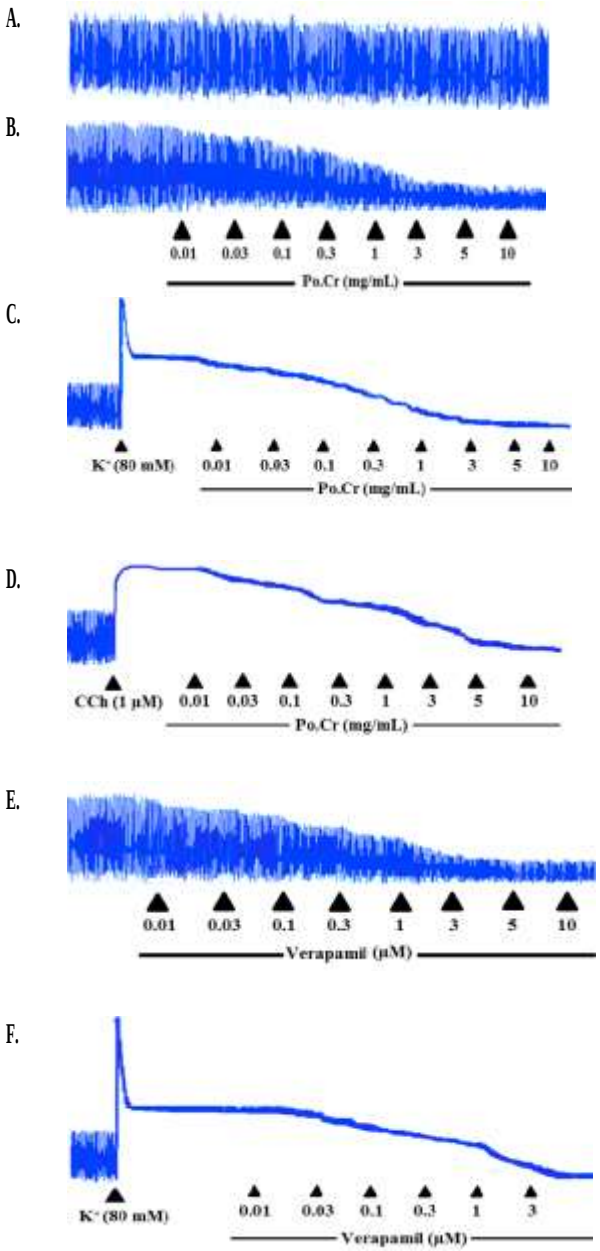

Fig. 1: Typical tracings showing (A) Control (B) the spasmolytic effect of crude extract of *Platanus orientalis* (C) K^+ (80mM) - induced contractions (D) carbachol (CCh: 1µM) - induced contractions (E) the spasmolytic effect of verapamil (F) verapamil on K^+ (80mM)-induced contractions on spontaneous contractions.

Table 1: Effects of crude extract of *Platanus orientalis* and verapamil on castor-oil induced diarrhea

Treatment	Dose	Total no. of feaces in 4h	Total no. of dry faeces in 4 h	Total no. of wet faeces in 4h	% Protection
Control	10 mL/kg	5.40 ± 0.51	5.20 ± 0.48	0.20 ± 0.20	96.68 ± 3.32
Castor oil	10 mL/kg	10.80 ±1.46	0.20 ± 0.2	10.60 ± 1.33	1.33 ±1.33
Po.Cr	100 mg/kg	8.20 ± 0.37	2.20 ± 0.2	6.00 ± 0.31	22.36 ± 4.43*
	300 mg/kg	8.00 ± 0.32	4.80 ± 0.37	3.20 ± 0.20	59.76 ± 2.87***
	1000 mg/kg	7.00 ± 0.55	5.60 ± 0.67	1.40 ± 0.24	79.20 ± 4.31***
Verapamil	10 mg/kg	7.00 ± 0.45	5.40 ± 0.24	1.40 ± 0.24	80.12 ± 2.97***

Table 2: Effect of Po.Cr extract on castor oil induced intestinal fluid accumulation.

Treatment	Dose	Intestinal Fluid Accumulation Pi/Pmx1000
Saline	10mL/kg	103.5±3.83
Castor oil	10mL/kg	154.0±4.30$^{\#}$
Po.Cr	100mg/kg	143.3±3.81*
Po.Cr	300mg/kg	123.5±9.29**
Po.Cr	1000mg/kg	95.30±1.35***
Loperamide	10mg/kg	84.20±2.61***

Mean±SEM (n=5),*p<0.05 vs saline, *p<0.05, **p<0.01, ***p<0.001 vs. castor oil

Table 3: Effects of Po.Cr extract on the gastrointestinal transit time in mice.

Treatment	Dose	Distance travelled by charcoal meal (%) ± S.D.
Control (saline)	5mL/kg	78.93 ± 6.629
Po.Cr	100mg/kg	67.21 ± 9.70
Po.Cr	1000mg/kg	50.66 ± 9.55***
Po.Cr	3000mg/kg	43.92 ± 10.97***
Loperamide	1mg/kg	61.33 ± 9.615**

Mean±S.D (n=5) **p<0.01, ***p<0.001 vs. control (saline)

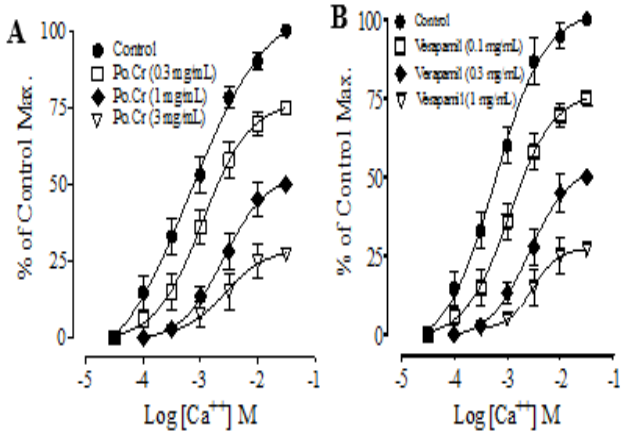

Fig. 2: $CaCl_2$ (Ca^{++}) in absence and presence of different concentrations of (A) crude extract of *Platanus orientalis* (B) verapamil in isolated mice jejunum preparations. Mean±SEM (n=5).

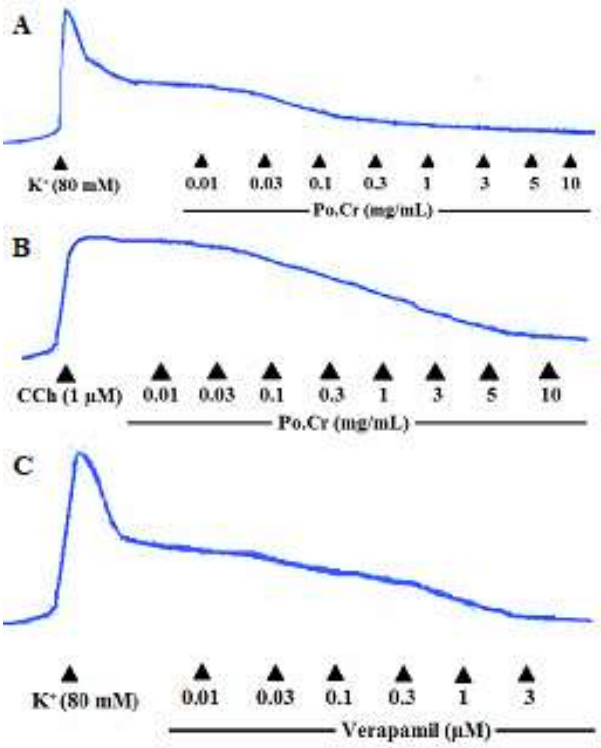

Fig. 3: Typical tracings showing the spasmolytic effect of crude extract of *Platanus orientalis* on (A) K^+ (80mM) - induced contractions (B) carbachol (CCh: 1µM) (C) verapamil on K^+ (80mM) - induced contractions in isolated mice ileum preparation.

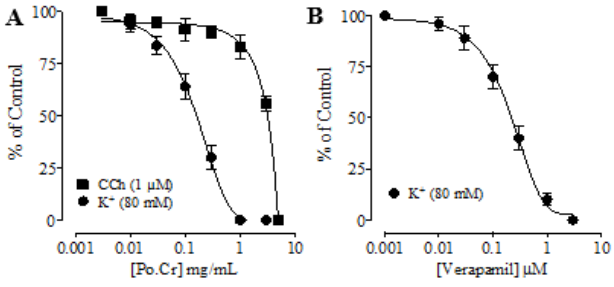

Fig. 4: Concentration-response curves of (A) Crude extract of *Platanus orientalis* on K^+ (80mM)- induced contractions and carbachol (CCh: 1µM)-induced contractions (B) verapamil on K^+(80mM)-induced contractions in isolated mice ileum preparations. Mean±SEM (n=5).

DISCUSSION

The crude extract of *Platanus orientalis* crude provided significant protection ($p<0.05$) in *in-vivo* experiments like verapamil and loperamide. Ricinoleic acid is a hydrolytic product of castor oil (used to induce diarrhea and enteropooling) which modifies water and electrolyte transport in intestine causing diarrhea, hypersecretions along with spasms in intestine (Jose *et al*., 2021) especially in transverse as well as distal colon (Croci *et al*., 1997). To see possible mechanisms involved in the inhibitory effects, *in-vitro* experiments were performed. Isolated jejunum of mice contracts spontaneously and allow studying effect of spasmolytic agents without use of an agonist or spasmogen. Calcium influx through voltage dependent calcium channels and discharge through sarcoplasmic reticulum results in the rise in the calcium levels which causes contractions in the jejunum tissues (Sagar *et al*., 2021). Crude extract of *Platanus orientalis* like verapamil which is a calcium channel blocker (Mapesa *et al*., 2021) caused inhibition of spontaneously contracting mice jejunum preparations. Previous literature revealed that most of plant extracts showed spasmolytic effects due to intestinal smooth muscle relaxation intervened by calcium channel blocking effect (Muhammad *et al*., 2021). The crude extract also inhibited contractions induced by high K^+ (80mM) in concentration dependent manner and the relaxation was found like verapamil as it exhibits such activity (Sagar *et al*., 2021), which suggest that it might be due to inhibition effect on calcium movement through VDCCs. Furthermore, *Platanus orientalis* crude extract like verapamil generated rightward shift in Ca^{++} CRCs (Elmongy *et al*., 2022) indicating the spasmolytic effect mediated through VDCCs. When mice jejunum preparations were tested against carbachol induced contractions, crude extract of *Platanus orientalis* exhibited spasmolytic effects indicating anticholinergic effects as carbachol is known to possess anticholinergic effect (Brown and Taylor, 2006; Saqib *et al*., 2021). Furthermore, the mechanism was further elucidated with the help of *In-Vitro* experiments performed on mice ileum tissue preparation suggesting the contribution of VDCCs and possibly muscarinic receptors antagonism. Crude extract of *Platanus orientalis* cleared acute toxicity tests hence found safe up to 5g/kg body weight.

CONCLUSION

Data indicates that crude extract of *Platanus orientalis* crude shows antidiarrheal, antisecretory and spasmolytic activities in mice though inhibitory effects on voltage dependent calcium channels (VDCCs) and muscarinic receptors. It provides a pharmacological base for the therapeutic importance of *Platanus orientalis* in these hyperactive gut disorders. However, further research is required for identifying chemical constituents and molecular nature of underlying mechanisms.

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