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COMMUNICATIONS

Effect of lapachol, a naphthaquinone isolated from *Tectona grandis*, on experimental peptic ulcer and gastric secretion

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Lapachol, a naphthaquinone isolated from the roots of *Tectona grandis* given at a dose of 5 mg kg^{-1} p.o. twice daily for 3 days was found to have an anti-ulcerogenic effect on subsequently induced experimental gastric and duodenal ulcers in rats and guinea-pigs. Its action appears to be associated with an effect on the protein content of gastric juice, and it reversed aspirin-induced changes in peptic activity, protein and sialic acid.

Parts of the common teak tree, *Tectona grandis*, are used in formulations to treat peptic ulcer in Indian medicine (Kirtikar & Basu 1944; Chunekar & Pandey 1969; Singh & Chunekar 1972). Pandey et al (1982) found that a 70% ethanol extract of the defatted trunk-bark and wood chips had a significant anti-ulcerogenic effect against experimental ulcers in albino rats and guinea pigs. In the course of the chemical examination of the root, trunk-bark and leaves, several compounds have been isolated and characterized. One of those from the powdered roots gave positive tests for quinones. On isolation by chromatographic resolution over silica gel it crystallized from benzene as bright yellow plates, $C_{15}H_{14}O_3$, mp. 139–40 °C, which from comprehensive spectral analysis and mixed melting point were identified as lapachol, as reported in the literature (Burnett & Thomson 1968; Sandermann & Simatupang 1968).

The IR spectrum showed bands at 3420 cm^{-1} for hydroxyl group and at 1600 cm^{-1} for the quinone carbonyl function. The ^{1}H NMR spectrum in CDCl$_3$ showed signals for two vinylic methyls at δ 1·54 and δ 1·75 (3H, *s* each) and allylic methylene at δ 3·25 (2H, *d*, J = 8 Hz) and olefinic hydrogens at δ 5·1 (1H, *m*) an exchangeable hydrogen at δ 7·3 (1H, *s*) and four aromatic hydrogens at δ 7·6 (2H, *m*, H-6 & H-7) and δ 8·01 (2H, *m*, H-5 & H-8). The mass spectrum of compound showed the molecular ion peak at *m/z* 242 (M$^+$) and other significant ion peaks at *m/z* 227 (base peak), 213, 209, 200, 199, 181, 179, 171, 159, 153, 152, 151, 143, 141, 131, 129, 128, 127, 116, 115 & 113. The ^{13}C NMR spectrum showed signals for two methyl carbons, two carbonyl carbons and ten other Sp^2 carbons in good agreement with the structure of lapachol. The resonance of different carbons are shown below.

Lapachol has now been studied for its effect on experimental ulcers and gastric secretion.

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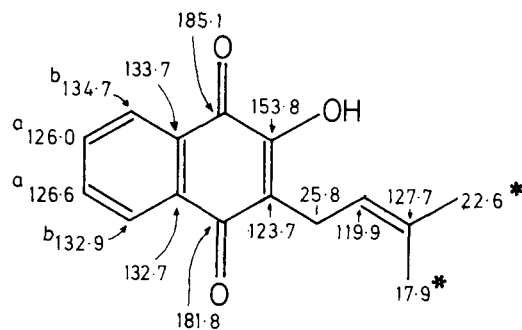
Structure of lapachol (a, b, * assignments are interchangeable).

Methods

Inbred albino rats of either sex, 120–140 g, and random-bred male guinea-pigs, 400–500 g, were used to study anti-ulcerogenic activity. They were housed in colony cages in a temperature-controlled (25 ± 2 °C) room with light and dark cycles of 10 and 14 h, respectively. The rats were fed Hind lever pellets, and guinea-pigs a pellet diet with cucumber, carrot and aubergine supplements until 18 h before the day of the experiment; animals had free access to water.

Gastric ulcers were produced in rats by (i) immobilization stress (Amar & Sanyal 1981), (ii) aspirin (0·2 g kg^{-1} p.o. for 4 days, the last dose being given 4 h before death) (Goel et al 1985) and (iii) in male guinea-pigs by a single injection of histamine acid phosphate (5 mg kg^{-1} i.p.) according to Eagleton & Watt (1965).

Duodenal ulcers were induced by cysteamine (30 mg kg^{-1} s.c., 18 h before death) in rats (Borella et al 1979; Gallagher & Szabo 1984) or by eight half hourly i.m. injections of histamine acid phosphate (0·25 mg kg^{-1}) in male guinea-pigs (Eagleton & Watt 1967).

Immobilization stress and aspirin-induced ulcers were scored as described by Goel et al (1985) and Sanyal et al (1982). Only the presence or absence of ulcers was noted in histamine-induced gastric and duodenal ulcers and in cysteamine-induced duodenal ulcers. Statistical analysis of data was by applying either Student's *t*-test or a Chi-squared test, as applicable.

Gastric secretion was collected in pylorus-ligated rats and the volume of filtered (through glass wool) gastric juice was measured and expressed in mL/100 g (Sanyal

et al 1971). Total acid was estimated by titration with 0.01 M NaOH against phenolphthalein and expressed as μ equiv/4 h. Peptic activity was determined by a modified method of Anson using haemoglobin as substrate (Debnath et al 1974) and expressed as μmol of tyrosine/4 h. Dissolved mucosubstances were estimated by determining the total carbohydrate (sum of total hexoses, hexosamine, fucose and sialic acid) and protein in the 90% ethanolic precipitate of gastric juice.

Table 1. Effect of lapachol on gastric ulcers induced by immobilization-stress (IS) and aspirin (ASP) and on duodenal ulcers induced by cysteamine in rats or histamine in guinea-pigs.

Treatment mg kg⁻¹ p.o. × days	No. of animals	Ulcer index Mean	s.e.m.	P value
				Student's t-test
Gastric ulcers				
Immobilization stress-induced ulcers (rats)				
Control	12	9·42	2·54	
Lapachol				
2 × 2·5 × 3	7	5·14	1·06	
2 × 5·0 × 3	12	2·33	1·34	<0·05
2 × 10.0 × 3	8	0·38	0·42	<0·01
Aspirin-induced ulcers (rats)				
Aspirin				
200 × 4	10	26·80	6·10	
Lapachol				
2 × 5·0 × 3				
+ aspirin				
200 × 4	8	7·13	1·92	<0·01
				Chi-squared test
Duodenal ulcers		No. with ulcers		
Cysteamine-induced ulcers (rats)				
Cysteamine				
30 s.c. × 18 h	10	8/10		
Lapachol				
2 × 5·0 × 3				<0·05
+ cysteamine				
30 s.c. × 18 h	10	2/10		
Histamine-induced ulcers (guinea-pigs)				
Histamine				
0·25 i.m. 8 inj/½ h	10	8/10		
Lapachol				
2 × 5·0 × 3				<0·05
+ histamine				
0·25 i.m. 8 inj/½ h	10	2/10		

The total carbohydrate and its ratio with protein (TC : PR) has been reported to serve as a reliable index of mucus secretion and mucosal resistance (Sanyal et al 1982, 1983).

Results and discussion

Initially, a dose-response study of the anti-ulcerogenic effect of lapachol was done in the immobilization stress-induced gastric ulcer model in albino rats. Significant protection was observed with a 5 and 10 mg kg⁻¹ p.o. dose given twice daily at 1000 and 1600h for 3 days followed by immobilization of the rats, previously fasted for 18 h, on day 4. There was no protection when the animals were given the above doses for 1 day only followed by immobilization on day 2. For further studies, therefore, a twice daily oral dose of 5 mg kg⁻¹ was given for 3 days. This treatment gave significant protection against all the experimental models of gastric and duodenal ulcers studied (Table 1) except histamine-induced gastric ulcers in guinea-pigs.

In the rats, lapachol did not significantly affect any of the secretory parameters except the protein content of gastric juice which it reduced significantly (Table 2). However, lapachol pretreatment significantly reversed aspirin-induced changes in peptic activity, protein and sialic acid content of the gastric juice. Thus, the TC : PR ratio, which was significantly decreased by aspirin, was reversed by· lapachol treatment (Table 2). These aspirin-induced changes in total hexoses and sialic acid content of gastric juice appear to be inconsistent, since under similar conditions, Goel et al (1985) did not observe them.

The findings suggest that the anti-ulcerogenic effect of lapachol might be primarily a result of the decrease in protein content in the gastric juice. The anti-ulcerogenic effect is five times more potent than 70% ethanolic extract tested earlier (Pandey et al 1982).

Table 2. Effect of lapachol (2 × 5 mg kg⁻¹ p.o. × 3 days) alone and on aspirin (200 mg kg⁻¹ p.o. × 3 days)-induced gastric secretion in pylorus-ligated albino rats.

Treatment	Volume mL/100g	Output/4 h Acid μ Equiv	Peptic activity μ mol	Protein (PR) μg mL⁻¹	Carbohydrate (μg mL⁻¹) Total hexoses	Hexos- amine	Fucose	Sialic acid	Total carb. (TC)	TC : PR
Control	1·93	171·3	762·4	605·2	364·4	205·6	76·4	38·0	684·3	1·18
	±0·31	±42·5	±126·8	±51·1	±33·9	±22·7	±16·6	±4·4	±53·1	±0·09
Lapachol	1·84	141·7	461·0	456·8*	294·9	179·2	63·2	35·7	573·6	1·36
	±0·44	±53·1	±85·7	±41·0	±23·6	±19·8	±8·1	±3·1	±50·8	±0·14
Aspirin	2·43	218·4	1028·2	791·4*	237·1**	201·8	55·1	24·6*	518·4*	0·74**
	±0·38	±42·2	+197·0	±63·8	±24·9	±29·8	±5·3	±2·4	±47·0	±0·08
Lapachol + aspirin	1·36†	107·2	534·6†	532·8†‡	315·2	224·6	73·9	35·7†	694·4	1·24†
	±0·33	±37·0	±119·6	±55·6	±39·0	±43·8	±10·0	±4·5	±69·6	±0·17

$n = 10$ in each group. Results are mean ± s.e.m.
* and ** indicate level of significance as $P < 0.05$ and < 0.01, respectively, compared with control group.
† and ‡ indicate level of significance as $P < 0.05$ and < 0.01, respectively, compared with aspirin group.

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Anticholinergic activity of the dopamine receptor agonist, TL-68 (N,N-dipropyl-2-aminotetralin)

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The anticholinergic properties of a dopamine receptor agonist, a non-hydroxylated derivative of N,N-dipropylaminotetralin (TL-68), were evaluated using the guinea-pig isolated tracheal strip and rat phrenic nerve-diaphragm preparations. TL-68 competitively antagonized carbachol-induced contractions in guinea-pig trachea with a pA_2 value of 5.88 ± 0.05. In the rat phrenic nerve-diaphragm preparation, TL-68 was found to be inactive in blocking nicotinic cholinergic receptors.

A dopamine receptor agonist, a non-hydroxylated derivative of N,N-dipropyl-2-aminotetralin (TL-68), produces central dopaminergic activity including emesis in dogs, inhibition of prolactin secretion in rats and contralateral rotation in unilaterally substantia nigra lesioned rats (Rusterholz et al 1979). The peripheral dopaminergic action of TL-68 was evaluated in cat hearts both in-vivo and in-vitro (Ilhan et al 1984).

Our preliminary experiments showed that TL-68 produced inhibition of twitch responses in transmurally stimulated guinea-pig ileum which was not antagonized by phentolamine, haloperidol or naloxone. These results led us to think about the postsynaptic anticholinergic action rather than the presynaptic inhibitory action of TL-68. The aim of this study was to get insight into the possible antimuscarinic and antinicotinic actions of TL-68 by using guinea-pig isolated trachea and rat phrenic nerve-diaphragm preparations.

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Materials and methods

Male guinea-pigs (300–500 g) were killed by a blow to the head. The excised trachea was cleaned of extraneous tissue and cut in a spiral fashion as described by Constantine (1965). The spiral was suspended in an organ bath (10 mL) containing Krebs-Henseleit solution and 2 g of resting tension was applied to the preparation.

The phrenic nerve-diaphragm preparation was prepared according to Bülbring (1946) from male rats (150–300 g). The left hemidiaphragms were suspended under 1 g of resting tension in 20 mL of Krebs-Henseleit solution. A bipolar platinum electrode was used for the phrenic nerve stimulation (indirect stimulation). The nerve was stimulated at a frequency of 0·05 Hz with supramaximal rectangular pulses of 0·1 ms duration. For direct stimulation of the diaphragm, a platinum electrode was attached to the muscle along its intercostal margin. An indifferent platinum electrode was immersed into the solution and supramaximal stimuli of 2 ms duration were applied at 0·05 Hz. The direct and indirect stimulations of the muscle were alternated at an interval of 10 s.

The composition of the Krebs-Henseleit solution was (mM): NaCl 118·4, KCl 4·69, MgSO$_4$ 1·18, CaCl$_2$ 2·5, KH$_2$PO$_4$ 1·17, NaHCO$_3$ 25·0, glucose 11·1. The solution was maintained at 33 °C and bubbled with a mixture of 95% O$_2$–5% CO$_2$.