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PHARMACODYNAMIC EVALUATION OF POLYHERBS AND ITS FORMULATION FOR ANTI-TUSSIVE ACTIVITY USING CITRIC ACID INDUCED IN GUINEA PIG

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ABSTRACT
This study was designed to evaluate the antitussive activity of Polyherbal extract containing Curcuma zedoaria rhizomes extract, Salvadora persica leaf extract, Glycyrrhiza glabra root extract, Andrographis paniculata leaf extract, Ocimum tenuiflorum leaf extract, citric acid Induced cough in guinea pigs. Six groups of guinea pigs (n = 6 in each group) were given the polyherbal formulation orally at a dose of 2000 mg/kg body weight for 14 consecutive days. On the first day, a cough was induced with citric acid, and the cough latency was measured in the first second and after 7 minutes, also used cytokine modulation.The latency to first cough was reduced from 13.3 s (disease control) when the linctus level was 3%, while the total number of coughs/7 min decreased from 12.5 to 5.1. The cough inhibition was 89% at 3% of linctus and 92% for dextromethorphan. Cumulative drug release in in-vitro diffusion study was found to be 94-98% at 16 hrs with maximum release achieved with the 3% linctus. Cytokine modulation showed decreases in TNF-α, IL-6 and IL-1β compared with disease control.The conclusion of the study shows that the prepared polyherbal linctus is pharmaceutically stable and has considerable antitussive activity almost equal to the standard treatment and can be used as a safe herbal alternative for cough and cold treatment.

KEYWORDS: Polyherbal linctus, Antitussive activity, Cytokine modulation, cough suppression.

1.INTRODUCTION

Patients with refractory chronic cough, which are frequently treated in specialized cough clinics, are the primary focus of the development of treatments for chronic cough. Asthma, chronic rhinosinusitis, and gastro-oesophageal reflux disease are often linked to chronic cough, which is common in respiratory illnesses.The most frequent cause of colds is rhinoviruses, which can cause nasal blockage, rhinorrhea, sneezing, sore throats, fever, headaches, and myalgia.

Stimulation of sensory receptors in the pharynx, larynx, trachea, and bronchi triggers the cough reflex by sending signals to the medullary cough center and triggering the respiratory muscles.Chemically sensitive C fibers and mechanically sensitive Aδ fibers are examples of airway sensory nerves. C fibers are triggered by irritants via TRPA1 receptors and by capsaicin via TRPV1 channels. Although it is still difficult to identify the primary receptors implicated in chronic cough, recent antitussive medications mainly target peripheral airway nerve receptors to block activation and prevent harmful central nervous system effects **[1,2].**

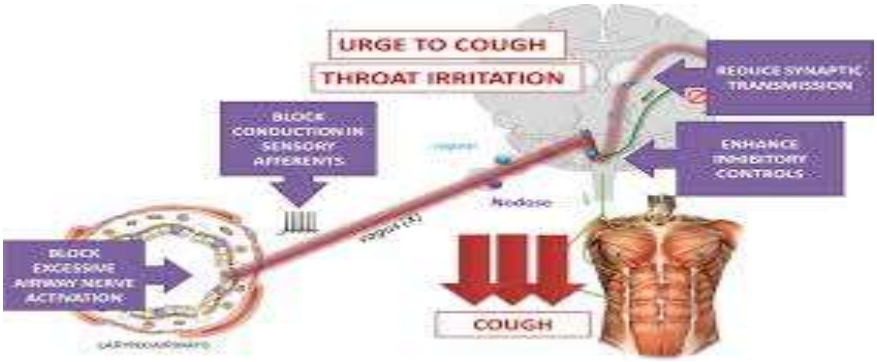


Fig 1: Mechanism of action of antitussive drugs [1,2]

2. MATERIALS AND METHODS

Curcuma zedoaria extract, Salvadora persica leaf extract, Glycyrrhiza glabra root extract, Andrographis paniculata leaf extract, Ocimum tenuiflorum leaf extract extracted by soxhlation process and Simple syrup (or glycerin–syrup base) and Sodium benzoate procured from our institute laboaratory.

2.1 Preparation of oral Linctus (medium strength, 20 mg/mL)

Accurately weigh 0.4 g each of standardized extracts of Curcuma zedoaria, Salvadora persica, Glycyrrhiza glabra, Andrographis paniculata, and Ocimum tenuiflorum (total 2.0 g). Dissolve the extracts in 20 mL of warm purified water and add gradually to the glycerin–syrup base with continuous stirring. Incorporate 0.1 g (0.1% w/v) sodium benzoate and flavoring agent q.s., make up the volume to 100 mL with purified water, mix gently, and fill into amber bottles labeled "Polyherbal Oral Linctus 20 mg/mL[3,4].

Table No.1 Formulation of polyherbal linctus

S. No.	Ingredient	Specification	Quantity
1	Curcuma zedoaria extract	Standardized dry extract	400 mg
2	Salvadora persica leaf extract	Standardized dry extract	400 mg
3	Glycyrrhiza glabra root extract	Standardized dry extract	400 mg
4	Andrographis paniculata leaf extract	Standardized dry extract	400 mg
5	Ocimum tenuiflorum leaf extract	Standardized dry extract	400 mg
Total herbal extract			2000 mg (2.0 g)
6	Simple syrup (or glycerin–syrup base)	Vehicle & sweetener	60 mL
7	Purified water	To dissolve extracts initially	≈ 20 mL
8	Purified water q.s.	To make final volume	q.s. to 100 mL
9	Sodiumbenzoate	Preservative (0.1% w/v)	0.1 gm
10	Flavoring agent	Flavour	q.s.

2.2 Combined FTIR Compatibility Data (Herbs + Excipients)

Fourier-transform infrared spectroscopy (FTIR) is a simple and effective technique for evaluating drug–excipient compatibility. Interactions are indicated by the disappearance, reduction, or emergence of absorption peaks. FTIR records spectra in the mid-infrared region (4000–400 cm^{-1}), where characteristic absorption bands identify specific functional groups based on molecular vibrational transitions[5, 6,7].

2.3 Cytokine Estimation

Cytokines are signaling proteins that regulate immune responses through receptor-mediated cell communication. In β-thalassemia major (β-TM), elevated levels of pro-inflammatory cytokines such as IL-6, TNF-α, and IFN-γ reflect persistent immune activation, oxidative stress, iron overload, and transfusion-related immune responses. Cytokine levels, including IL-4, IL-6, IL-8, IL-13, and TGF-β, are commonly measured in serum or plasma using enzyme-linked immunosorbent assay (ELISA)[13].

2.4 In Vitro Diffusion Study of Herbal Linctus

The rate at which the active ingredient in herbal remedies is released into dissolution media is assessed using in vitro dissolution studies.The dissolve test verifies the formulation's integrity and shows how bioavailable poorly soluble herbal remedies are.The linctus is placed in a USP dissolving apparatus with physiologically acceptable media (pH 1.2, 4.5, and 6.8) that is maintained at 37 ± 0.5 °C.[15]

2.5 Evaluation of polyherbal linctus

2.5.1 Appearance & Organoleptic properties

Pour a little of the linctus into a clean glass and compare it on a black and white background. Inspect for the color, clarity, smell, taste and possible presence of suspended particles/lumps.

2.5.2 PH determination

Use standard buffers (pH 4.0 and 7.0) to calibrate a pH meter. Insert glass electrode in the sample of linctus at 25 °C and let the reading stabilize [8,9].

2.5.3 Viscosity

Use a Brookfield viscometer, using the right spindle. Place sample into beaker, dip spindle, set rotation speed and record the reading once the spindle has settled.

2.2.4 Specific gravity

Clean and dry a pycnometer, weigh empty (W_1). Top with linctus, remove air bubbles and weigh (W_2). Specific gravity = $(W_2 - W_1) / (W_3 - W_1)$.

2.2.5 Refractive index

Carefully add 1-2 drops of linctus to the prism of a refractometer, incubate at 25 °C, close the refractometer and read when the bou ndary line becomes sharp [10].

2.2.6 Total solids content

Accurately measure 5 mL linctus into a pre-weighed evaporating dish. Dry on a water bath and in an oven hot air at 105 °C (if necessary, correct the density), cool and weigh. % Total solids = (Weight of residue/volume taken × density correction if necessary) × 100.

2.2.7 Assay of herbal extract content (marker estimation)

Make appropriate concentrations of linctus. Determine by UV–Visible spectrophotometry and/or HPLC by using suitable marker compounds such as andrographolide, glycyrrhizin, and curcuminoids. Compare with the calibration curve of the standards [11].

2.2.8. Microbial load

Total aerobic plate count of nutrient agar and Sabouraud agar (for fungi) by pour-plate or spread-plate method. Incubate plates (bacteria 37 °C/24–48 h; fungi 25 °C/5–7 days). Absence of specified pathogens and counts should be within pharmacopeial limits.[12]

2.2.9. Sedimentation / physical stability

Store samples at room temperature and at accelerated conditions (40 °C ± 2 °C) for 1 - 3 months. Look for sedimentation, crystallization, changes of colour, changes of smell and phase changes every so often.[13]

2.6 Pharmacological studies

2.6.1 Procurement of experimental animals

Guinea pigs (700–1200 g, either sex) were obtained from the Central Animal Facility, NIIMS University Institute of Pharmacy, India. The study was approved by the Institutional Animal Ethics Committee (IAEC Protocol No. 1/NIIMS/IAEC/2024, dated 01/09/2025). Animals were housed under standard conditions with a 12 h light/dark cycle, pellet diet, and water ad libitum, acclimatized for one week, and treated orally using a gavage tube.[16]

2.6.2 Acute toxicity study

2.6.2.1 LD_{50} & ED_{50} / Tolerance dose determination

The ED_{50} value measures a drug's potency relative to a reference standard for graded dose–response relationships. For quantal (all-or-none) responses, the corresponding parameter is the LD50 value. Together, ED50 and LD50 are important indicators for evaluating a drug's efficacy and safety.[17].

The ratio between LD_{50} and ED_{50} represent the therapeutic index. Greater the therapeutic index, safer the drug.

Animal	:	Guinea pigs
Number of animal	:	6
Sex	:	Male/Female
Age and Body weight	:	10 weeks, 700-1200 g
Sample	:	Extract* of Curcuma Zedoria, Glycyrrhiza glabra Salvadora persica, Andrographis paniculata and Ocimum tenuiflorum
Dose	:	2000mg/kg body weight
Route of administration	:	Oral
Duration observation	:	10-14 days

The guinea pigs were checked for toxicological symptoms at 0-0.5, 0.5-1, 1-2, 2-4, and 48 hours after dose. Each Guinea pig's body weight was measured before the test medication was administered as well as seven and fourteen days later [12]. The research was carried out in accordance with OECD Test Guideline 425, Limit Test at 2000mg/kg (Revised: 17 December 2001).

2.6.2.2 Experimental Design

Guinea pigs were used in the evaluation of antitussive activity of the herbal linctus (causing cough by citric acid) in six experimental groups [18].

Table 4: Animal Grouping and Treatment Protocol

Group	Treatment	Details / Dose
I. Normal Control	Vehicle only	No citric acid exposure; baseline cough response
II. Disease Control	Citric acid + vehicle	7.5% w/v citric acid aerosol for cough induction
III. Standard Control	Citric acid + Dextromethorphan	10 mg/kg p.o. (reference antitussive drug)
IV. Test Low Dose	Citric acid + Herbal Linctus 1%	1% w/v suspension p.o.
V. Test Medium Dose	Citric acid + Herbal Linctus 2%	2% w/v suspension p.o.
VI. Test High Dose	Citric acid + Herbal Linctus 3%	3% w/v suspension p.o.

2.7 Histopathology Study

Histopathological analysis involves fixing tissues in 10% neutral buffered formalin, paraffin embedding, sectioning (4–5 μm), and staining with hematoxylin and eosin (H&E) or specialized methods. Proper tissue processing ensures high-quality sections for accurate microscopic evaluation of pathological changes [17, 18,19].

2.8 Statistical analysis

All data is expressed using the mean ± SEM (Standard Error of Mean). The statistical analysis employed Dunnett's post-hoc test for multiple comparisons and one-way ANOVA . A value of $p < 0.05$ was considered statistically significant.

3. RESULT

Table :2 Physicochemical parameters of formulated herbal linctus

S. No.	Parameter	Observed Result	Remark
1	Appearance	Uniform, clear, viscous brown liquid	Complies
2	Odour	Characteristic, pleasant herbal odour	Complies
3	Taste	Sweet, acceptable with mild bitterness	Complies
4	pH	5.6 ± 0.1	Within acceptable oral range
5	Viscosity	1850 cP at 25 °C	Characteristic of linctus
6	Specific gravity	1.24	Within expected range
7	Refractive index	1.412 at 25 °C	Normal for syrupy liquids
8	Total solid content	64% w/v	Acceptable
9	Assay of total extract content	19.6 mg/mL (≈ 98% of label claim)	Acceptable
10	Microbial load	Within pharmacopeial limits; no pathogens detected	Pass
11	Sedimentation/Physical stability	No sedimentation or phase separation after storage	Stable
12	Content uniformity	±4.5% variation among samples	Acceptable
13	Container compatibility	No change in container or product characteristics	Compatible

3.1 In Vitro Diffusion Study of Herbal Linctus

In vitro dissolution studies evaluate the release of active phytochemicals from the linctus and assess formulation performance. The linctus is tested using a USP dissolution apparatus in media of pH 1.2, 4.5, and 6.8 at 37 ± 0.5 °C. Samples are collected at predetermined intervals, analyzed by UV–Vis spectrophotometry, and the cumulative percentage of drug released is plotted against time.

Table:3% Cumulative linctus release of polyherbal formulation

Time (in Hrs.)	HL-I (1%)	HL-II (2%)	HL-III (%)
0	0 %	0 %	0 %
1	15.26 %	14.82 %	18.15 %
2	23.87 %	27.15 %	25.63 %
3	30.12 %	30.14 %	31.25 %
4	35.95 %	32.95 %	33.56 %
5	40.12 %	39.01 %	42.03 %
6	51.23 %	48.95 %	53.6 %
7	57.36 %	50.23 %	58.2 %
8	63.14 %	59.87 %	64.74 %
9	70.14 %	67.71 %	71.36 %
10	78.1 %	74.82 %	79.51 %
11	82.16 %	80.09 %	83.02 %
12	89.25 %	84.05 %	90.08 %
13	92.12 %	86.07 %	93.54 %
14	93.56 %	91.08 %	94.1 %
15	94.12 %	94.22 %	96.52 %
16	97.22 %	97.98 %	98.4 %

***HL – Herbal Linctus**

Table : 4 Statistical Values of Formulations

Formulation	Mean ± SD (%)
HL-I (1%)	59.06 ± 33.48
HL-II (2%)	57.95 ± 33.58
HL-III (3%)	60.69 ± 33.73

Mean ± SEM one way ANOVA and P value significance **(P<0.05)**

Formulations HL-I, HL-II, and HL-III showed a time-dependent increase in cumulative drug release, reaching 97.22%, 97.98%, and 98.40%, respectively, at 16 h (mean ± SD, n = 3). Among the formulations, HL-III exhibited the highest cumulative drug release.

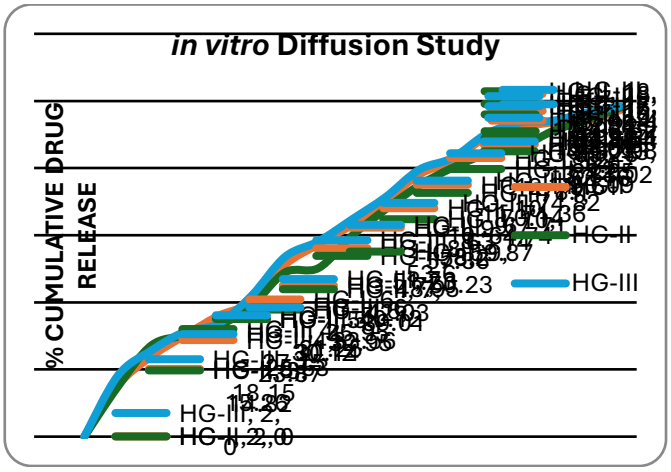


Figure :1 In vitro Drug diffusion study of formulated Linctus

3.2 Pharmacological Evaluation of Antitussive Activity (Citric Acid-Induced Cough Model)

Table: 5 Latency to first cough (seconds)

Group	Treatment	Dose	Latency (sec) (Mean ± SEM)	% reduction in cough but against control
I	Normal Control	Vehicle	—	—
II	Disease Control	Citric Acid + Vehicle	13.3 ±1.88	85
III	Standard	Dextromethorphan 10 mg/kg	8.80 ±0.82	45
IV	Test Low Dose	Herbal Linctus 1%	7.32 ±1.25	54
V	Test Medium Dose	Herbal Linctus 2%	4.99 ±1.06	70
VI	Test High Dose	Herbal Linctus 3%	4.00 ±0.81	75

A significant decrease in cough latency was observed in the standard and Herbal Linctus-treated groups compared with the disease control group. The maximum antitussive effect was observed with Herbal Linctus (3%), showing a 75% reduction in cough, which was comparable to the standard drug Dextromethorphan.

Table: 6 Total Number of Coughs (7 minutes)

Group	Treatment	Total Coughs (Mean ± SEM)
I	Normal Control	0
II	Disease Control	12.5 ± 1.3
III	Standard	6.2 ±0.5
IV	Herbal Linctus 1%	9.2 ±0.9
V	Herbal Linctus 2%	7.6±0.7
VI	Herbal Linctus 3%	5.1±0.5

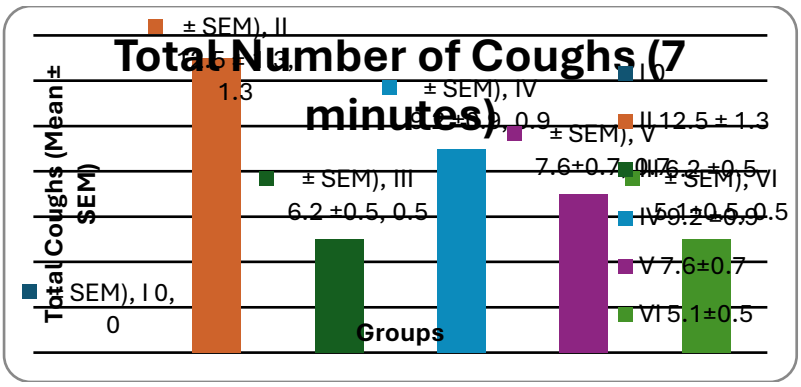


Figure :3 Total Number of Coughs (7 minutes)

Treatment with the standard drug significantly reduced the number of coughs to 6.2 ± 0.5, corresponding to 50.4% inhibition. Herbal Linctus produced a dose-dependent antitussive effect, with cough counts decreasing to 9.2 ± 0.9, 7.6 ± 0.7, and 5.1 ± 0.5 for the 1%, 2%, and 3% formulations, respectively.

Table:7 Percentage Inhibition of Cough (%)

Group	Treatment	% Inhibition
I	Normal Control	—
II	Disease Control	0%
III	Standard	92%
IV	Herbal Linctus 1%	62%
V	Herbal Linctus 2%	78%
VI	Herbal Linctus 3%	89%

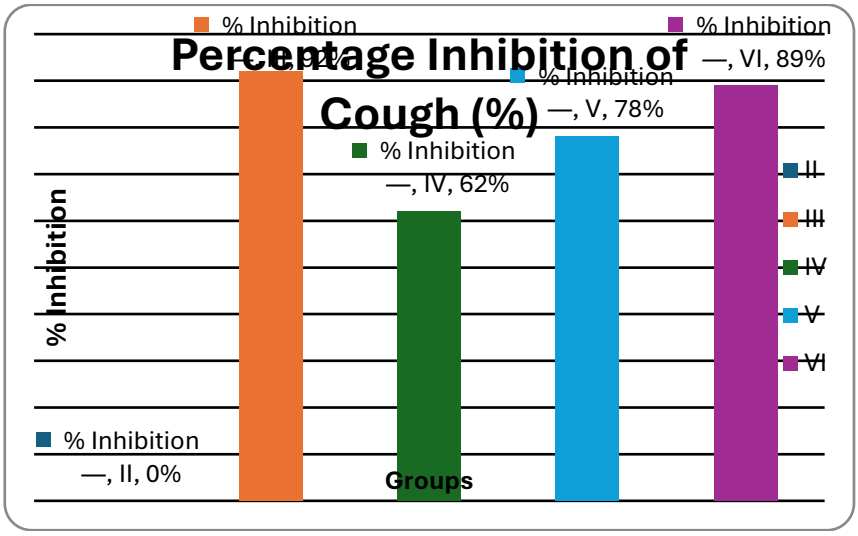


Figure :4 Percentage Inhibition of Cough (%)

Data were expressed as mean ± SEM (n = 6) and analyzed using one-way ANOVA followed by Dunnett's test. Statistical significance was set at $P < 0.05$. The standard (92%) and HL 3% (89%) showed highly significant effects ($P < 0.001$), HL 2% (78%) was significant ($P < 0.01$), and HL 1% (62%) showed moderate significance ($P < 0.05$).

3.3 Cytokine Estimation

Standard ELISA kits were used to measure the levels of pro-inflammatory cytokines such TNF-α, IL-6, and IL-1β. Effect of polyherbal linctus on citric acid-induced inflammatory condition of airways was assessed by the inflammatory markers .

Table: 8 Cytokine Levels (Mean ± SEM)

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)
Group I: Vehicle Control	0	0	0
Group II: Disease Control	32.4±2.1	18.6±1.3	55.2±2.8
Group III: Standard (DXM 10 mg/kg)	88.7±4.7	64.3±3.4	28.6±2.1
Group IV: Herbal Linctus 1%	41.2±3.2	31.4±2.2	48.1±2.6

Group V: Herbal Linctus 2%	55.3±3.0	40.8±2.6	41.7±2.0
Group VI: Herbal Linctus 3%	43.9±2.7	35.2±2.1	47.9±2.3

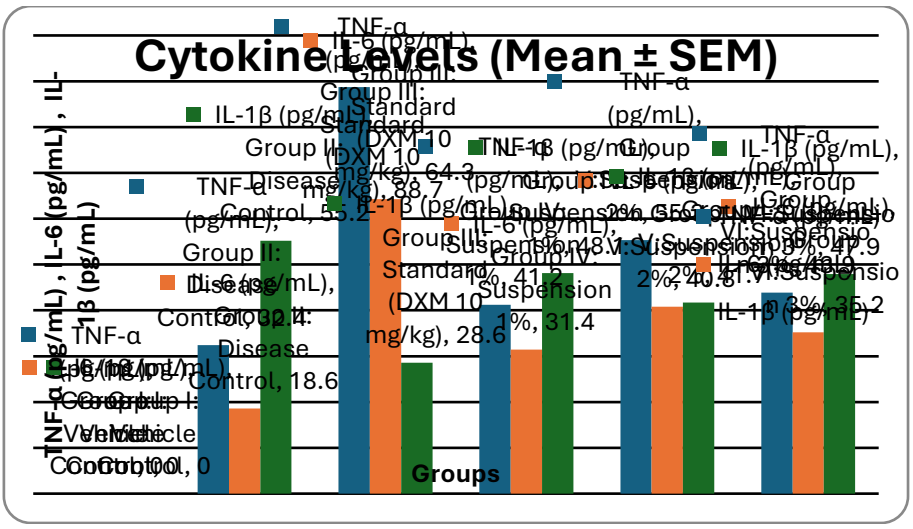


Figure :5 Cytokine Levels (Mean ± SEM)

Herbal linctus reduced inflammatory cytokines in a dose-dependent manner. The 2% formulation showed the greatest effect, lowering TNF-α, IL-6, and IL-1β to 55.3 ± 3.0, 40.8 ± 2.6, and 41.7 ± 2.0 pg/mL, respectively, indicating significant anti-inflammatory activity compared with the disease control group.

3.4 Combined FTIR Compatibility Data (Herbs + Excipients)

FTIR analysis of the polyherbal formulation confirmed the presence of characteristic phytochemical functional groups. A broad absorption band at 3350–3420 cm^{-1} indicated hydroxyl groups of phenols, alcohols, and flavonoids, while alkane and lipid-associated bands were observed in all samples, confirming the phytochemical composition of the selected plant extracts.

Table: 11Combined FTIR Compatibility Data (Herbs + Excipients)

Functional Group	Wave number (cm^{-1})	Curcuma zedoaria	Salvadora persica	Glycyrrhiza glabra	Andrographis paniculata	Ocimum tenuiflorum	Glycerin–Syrup Base	Sodium Benzoate
OH stretching	3200–3500	3420	3350	3400	3360	3355	3300–3400	—
C–H stretchin	2850–2960	2925	2920	2930	2925	2960	2850–2950	—
C=O stretchg	1650–1750	1735	1650	1715	1675	—	—	~1600 (COO^-)
Aromatic C=C	1500–1625	1625, 1510	—	1600	—	1640, 1515	—	1450–1500
N–H bending	~1540	—	1540	—	—	—	—	—
C–H bending	1380–1455	—	1450	1380	1455, 1385	—	—	—
C–O stretchg	1000–1270	1270, 1025	1100	1075	1050	1265, 1030	1000–1150	—
COO^- symmetric	~1400	—	—	—	—	—	—	~1400
Aromatic C–H bending	700–900	—	—	—	—	—	—	700–900

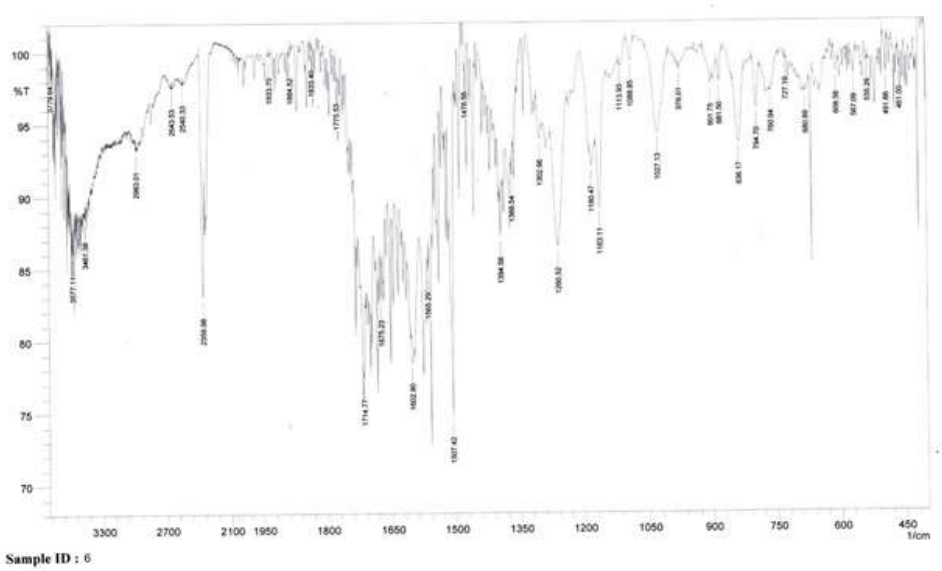


Fig: 6 Combined FTIR Compatibility Data (Herbs + Excipients)

FTIR spectra confirmed the presence of O–H, C=O, C=C, and C–O functional groups, indicating polyphenols, glycosides, and other phytoconstituents. Sodium benzoate showed characteristic COO^- peaks without interfering with other components. The combined formulation retained all characteristic peaks with no significant shifts, confirming herb–excipient compatibility and the absence of chemical interactions.

3.5 Histopathology Study

3.5.1 Disease Control (Group I)

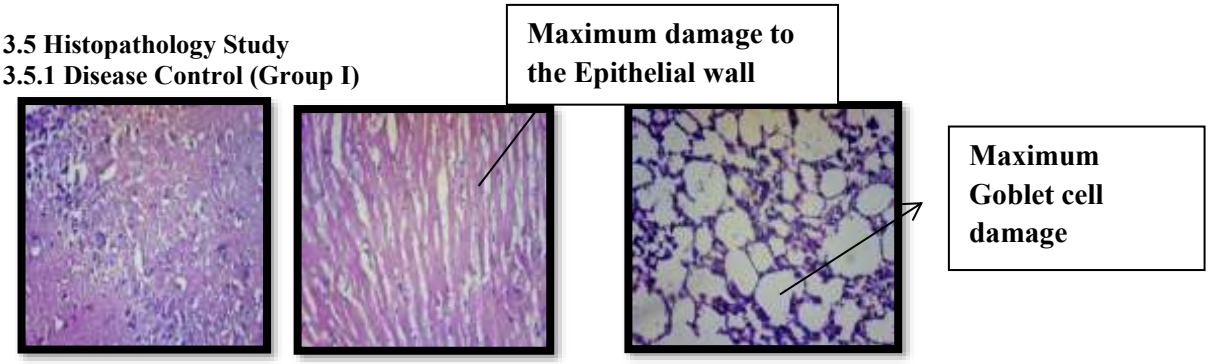


Figure 1: Disease Control (Group I) shown Severe bronchial epithelial damage , Inflammatory cell infiltration (neutrophils, macrophages), Submucosal edema , Goblet cell hyperplasia → excess mucus and Vascular congestion.

3.5.2 Standard Group (Dextromethorphan)

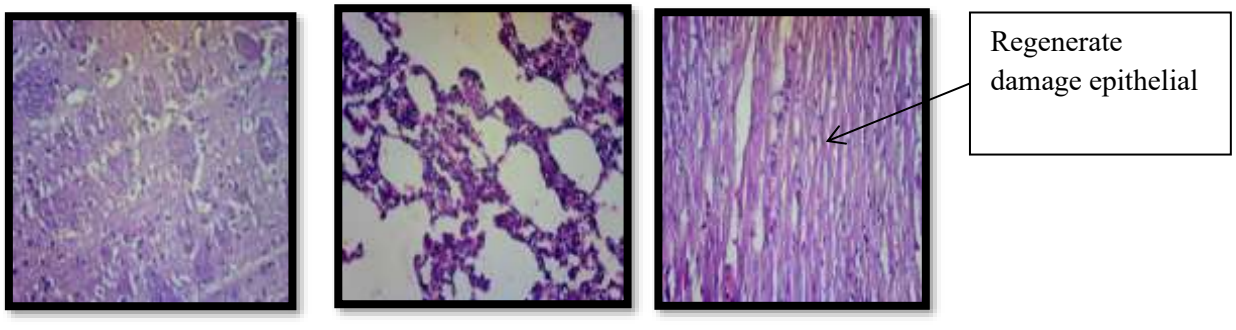
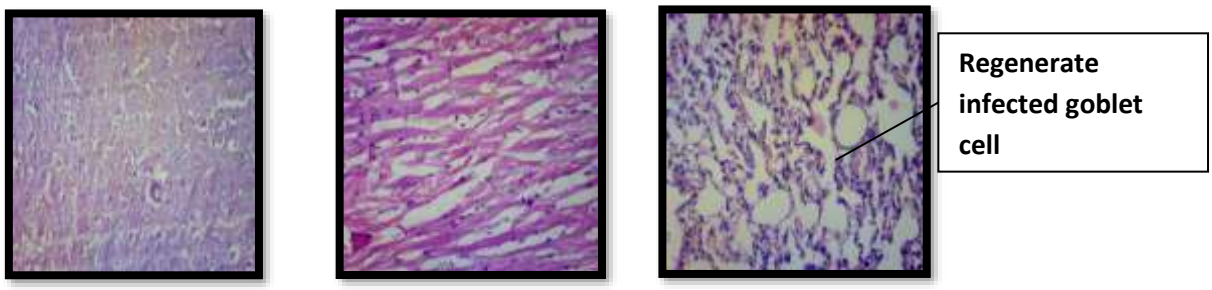


Figure 2: Standard Group (Dextromethorphan) shown Mild inflammation , Reduced epithelial disruption , Near-normal airway structure and Minimal mucus secretion

3.5.3 Herbal Linctus Groups (Dose-Dependent Recovery)



(A) (B) (C)

Figure 3: Herbal Linctus Groups Near-normal bronchial architecture, Minimal inflammation and Significant reduction in goblet cells

4.DISCUSSION

The current study showed that the prepared herbal linctus has a strong antitussive effect in guinea pigs that is clearly dose-dependent. The successful induction of the cough model was confirmed by the disease control group's severe cough bouts.The release of active components gradually increased as the concentration of herbal extracts increased, according to in vitro drug diffusion tests conducted for HL 1%, HL 2%, and HL 3% formulations.HL 3% had the highest diffusion rate of the three formulations, suggesting improved release properties and increased bioactive component availability.The optimized phytoconstituent content and the glycerin-syrup base's compatibility, which promoted effective drug release and dispersion, may be responsible for the enhanced diffusion profile of HL 3%.Cytokine research provided additional proof of the herbal linctus's anti-inflammatory properties.

When compared to the disease control group, all treated groups showed a significant decrease in interleukin-6 (IL-6), a key pro-inflammatory cytokine involved in respiratory inflammation.But when compared to HL 1% and HL 2%, the HL 3% formulation showed the largest percentage inhibition of cough and the biggest increase in cough latency, indicating higher efficacy. The combinatorial impact of many phytoconstituents that act on various pathways involved in cough production and inflammatory processes may be the cause of the increased antitussive activity seen with HL 3%.

Additional proof of the herbal linctus's medicinal effectiveness came from histopathological analysis of lung tissues. Significant pathological changes, including tissue congestion, inflammatory cell infiltration, goblet cell hyperplasia, and epithelial cell destruction, were observed in the disease control group. Treatment with the herbal formulations, on the other hand, significantly decreased these pathogenic alterations.

5.CONCLUSION

The proposed grouping is to determine if there is a dose-response relationship between the herbal linctus antitussive effect and the lower formulations. The 3% formulation demonstrated a stronger antitussive effect than the lower formulations and was comparable to that of the conventional medicine. The test doses (1%, 2%, 3%) were selected as a result of the preliminary phytochemical analysis and preliminary tests that were conducted to determine the efficacy of the doses over time. To find the best therapeutic benefit while maintaining safety and tolerability in test animals, the highest dose (3%) was used. It is expected that the number of citric acid-induced cough episodes will be significantly reduced; the response pattern would be: 3% > 2% > 1% > Disease control and approaching the activity of Dextromethorphan. Overall cough frequency was significantly reduced and latency to first cough was increased with the 3% herbal linctus compared to the disease control ($p < 0.05$). Of all the test concentrations, the 3% dose was most effective in reducing cough by 95%, just slightly less than the usual cough medicine.

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