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Antihistamine from *Tragia involucrata* L. leaves

Abstract

Background: Synthetic antihistamine drugs cause various adverse effects to overcome these problems with natural phytomedicine or phytoconstituents.

Methods: *Tragia involucrata* leaves were extracted with soxhlet apparatus and fractionated with column chromatography the homogenized fractions were monitored with thin layer chromatography (TLC) and characterized by using UV-visible, FT-IR, ^1H NMR, ^{13}C NMR and MS spectral studies. Isolated compounds were screened their antihistamine activity on ileum preparation, bronchoconstriction and triple response on histamine-induced guinea pig.

Results: Antihistamine 5-hydroxy-1-methylpiperidin-2-one has been isolated and characterized from the leaves of *Tragia involucrata* L. A promising muscle relaxant, bronchorelaxant and anti-allergic effect of 5-hydroxy-1-methylpiperidin-2-one was observed in histamine-induced guinea pig and found to be $55.54 \pm 2.78\%$ protection at the dose level of 12.5 mg/kg in bronchoconstriction effect and $49.05 \pm 2.45\%$ protection in triple response. These findings were confirmed by *in silico* molecular docking also against histamine H_1 receptor compared with chlorpheniramine maleate and mepyramine. This shows that the 5-hydroxy-1-methylpiperidine-2-one possess good inhibitory effect on histamine-induced guinea pig. The muscle relaxant, bronchodilating and anti-allergic potency of 5-hydroxy-1-methylpiperidin-2-one has been discussed in context with its probable profile as an anti-asthmatic agent from *T. involucrata* L. leaves.

Conclusions: We can conclude that isolated 5-hydroxy-1-methylpiperidin-2-one from *T. involucrata* L. has potent antihistamine agent on histamine-induced guinea pig.

Keywords: antagonist, antihistamine, bronchoconstriction, histamine H_1 receptor, *Tragia involucrata* L., triple response

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Introduction

Asthma is a chronic inflammatory lung disease due to the airway irritants like tobacco smoke, air pollution, allergens, respiratory infections, stress, mold, termites and genetic and environmental factors. The present treatment methods and resources for allergic disease such as asthma have low effectiveness, related to adverse effects and subjected to fulfillment [1]. Plant is a major source for human being as a natural medicine and it saved their lifetime from various diseases with their bioactive properties used in the modern medicine [2, 3]. The allergens, one of the most causative agents of asthma, produce histamine and leukotriene on mast cell and basophils when inhaled through respiration. Histamine induces bronchoconstriction mainly mediated through the histamine H_1 receptor [4], produces pruritus, vasodilatation, hypotension, flushing, headache, tachycardia and bronchoconstriction. The chlorpheniramine maleate (CPM) is the most common antihistaminic drug in the market.

Histamine, one of the most important molecules, is a key mediator in allergic rhinitis (AR). It is an inherent vasoactive property released from granules contained within mast cells, basophils, lymphocytes and other reservoirs and interacts with histamine receptors. The H_1 -histamine receptor is associated with modulation of proinflammatory immune cell activity [5] and its interaction with histamine is the prime focus of suppressive therapy for AR. The synthetic anti-asthmatics are diphenhydramine, chlorpheniramine, mepyramine, promethazine, loratadine that have various adverse effects such as drowsiness, dry mouth, constipation, confusion, nightmares, nervousness, restlessness and irritability. In view of that, the present work showed anti-asthmatic and anti-allergic activity of the phytoconstituents present in the methanol extract of *Tragia involucrata* L. (Euphorbiaceae) leaves by *in vitro* and *in vivo* and *in silico* approach. The extract of *T. involucrata* L. already reported that their active mechanism is against various diseases such as anti-inflammatory,

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analgesic activity [6], acute and subacute inflammation. It is also used for bronchial asthma as herbal medicine in the form of decoction [7], the *T. involucrata* L. combination with black pepper to cure asthma [8] and it is used in the Ayurvedic formulation of Pankajakasthuri.

Materials and methods

Collection of plant material

T. involucrata L. leaves were collected at Kolinjamparai, Palakkad District, Kerala, India, on winter season and the specimen was authenticated and identified by Dr G. V. S. Murthy, Scientist “F”, Botanical Survey of India, Coimbatore 641 003, Tamil Nadu, India (Vide No. BSI/SRC/5/23/2010-11/Tech-1848 dated 8 February 2011), and the voucher specimen was deposited at the same institute for future reference.

Instruments

Aerosol chamber with central spout for introduction of atomized histamine, atomizer, vacuum pump, stop watch and disposable syringe and needle (facility provided by Karpagam College of Pharmacy, Coimbatore). Column chromatography, UV-visible spectrophotometer, Fourier transform infrared spectroscopy (KUIF, Karpagam University, Coimbatore), mass spectrometry and nuclear magnetic resonance (SAIF-STIC, CUSAT, Cochin, Kerala).

Chemicals

Histamine disulfate from HiMedia Laboratory, CPM from Unimark Remedies Ltd., Bangalore. All the chemicals used in this study including the solvents were of analytical grade.

Animals

Guinea pigs (250–350 g) of either sex were housed for 2 weeks prior to the experiment for acclimatization in the animal house of Karpagam University Animal House, Coimbatore, Tamil Nadu, India. Animals were maintained under controlled conditions of temperature $26 \pm 2^\circ\text{C}$, relative humidity 44–56% and photo-schedule (12 h light and 12 h dark). Animals were provided with standard diet and water *ad libitum*. The food was withdrawn 18 h before the start of the experiment. Institutional Animal Ethics Committee approved the experimental protocol (739/03/abc/CPCSEA). The pharmacological work was carried out as per norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Extraction and fractionation

The collected plant materials (leaves) were washed thoroughly in tap water, chopped, air dried for 2–3 weeks at $35\text{--}40^\circ\text{C}$ and pulverized in electric grinder. The 500 g dry powder obtained after defatted with

petroleum ether and successively extracted with methanol ($64\text{--}66^\circ\text{C}$) using soxhlet apparatus, finally obtained 20 g of extract in the semi-solid form [9]. The same was impregnated with silica gel and loaded onto top of the silica gel (mesh size $60\text{--}120\ \mu\text{m}$) column ($1000\ \text{mm} \times 40\ \text{mm}$) as stationary phase. Based on thin layer chromatography (TLC) experiments, the mobile phase was selected as petroleum ether–ethyl acetate and chloroform–methanol mixture to elute with increasing polarity of the solvents. The uniform quantities of the different fractions (25 mL) have been collected and monitored by TLC. Iodine was used as the detecting agents for colorless compounds. Similar fractions were mixed together for further analysis and then it is subjected to elucidate the chemical structure.

Isolation and characterization

Fractions of 25 mL have been collected and monitored by TLC and homogenous fractions were taken to elicit the structure by using spectral studies. The spectral data of following instruments were used to elucidate the chemical structure with UV Visible spectrophotometer (UV-Vis-2450, Shimadzu, Japan) for λ_{max} value, FT-IR (IR Affinity-1, Shimadzu, Japan) for functional group analysis, ^1H NMR (Bruker DRX 400 instruments $399.95\ \text{MHz}$) for number of proton signals present in the molecule and ^{13}C NMR (Bruker AMX 300–75.5 MHz) for number of carbon atom present in the molecule and mass spectral data (Jeol GC Matell) to determine the molecular weight of the molecule [10].

Evaluation of antihistamine activity of isolated compound 1

Isolated guinea pig ileum preparation: Male albino fasted (24 h) guinea pigs weighing 250–350 g were killed by a blow to the head and exsanguinated. Terminal segments of ileum about 1–1.5 cm in length were prepared and placed in 30 mL baths filled with Tyrode solution (NaCl , 136.7; KCl , 2.68; MgCl_2 1.05; NaH_2PO_4 0.42; CaCl_2 , 1.80; NaHCO_3 , 11.90; glucose, 5.55 mM). The solution was kept at 37°C and oxygenated continuously. Initial tension was 1 g and stabilization time was 45–60 min. Isometric contractions were recorded on NARCO F-60 transducer connected to a NARCO trace 80 recorder. After stabilization of organ bath, increasing concentrations of histamine were added to the bath and the control cumulative concentration–response curve for each one (histamine) was constructed. Isolated compound 1 from *T. involucrata* L. leaves, CPM was then added to the bath 1 min before the corresponding concentration response curve was recorded [11].

Histamine-induced bronchoconstriction in guinea pigs

Overnight fasted guinea pigs were divided into four groups ($n=4$): Inducer (IC) = histamine (0.2%, aerosol), STD received CPM (2 mg/kg, i.p.), compound 1 (10.0 mg/kg) and compound 1 (12.5 mg/kg). Bronchoconstriction was induced in guinea pigs by exposing them to histamine aerosol (0.2%) produced by an ultrasound nebulizer in an aerosol chamber ($24 \times 14 \times 24\ \text{cm}$) made of perspex glass. The time required for appearance of pre-convulsive dyspnea caused by the histamine was recorded for each animal. Prior to drug treatment, each animal was placed in the histamine chamber

and exposed to 0.2% histamine aerosol. The pre-convulsion time (PCT), the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion, was noted. As soon as the pre-convulsion dyspnea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover. This time for pre-convulsive dyspnea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnea for 4 h. After 4 h, the animals received compound 1 and CPM. These animals were again subjected to histamine aerosol later at an interval of 30 min, 1 h and 4 h to determine PCT [12]:

The protection offered by the treatment was calculated using the formula Percentage protection = $(1 - T_1/T_2) \times 100$

where T_1 = the mean of PCT before administration of test drugs and T_2 = the mean of PCT after administration of test drugs at 30 min, 1 h and 4 h.

Histamine-induced triple response in guinea pig

Experiments were performed with overnight fasted guinea pig, 250–350 g body weight was divided into four groups ($n=4$): inducer (IC) = histamine (0.2%, aerosol), STD received CPM (2 mg/kg, i.p.) and compound 1 (12.5 mg/kg) were used for inducing the allergic reactions as triple response are red spot, flare and wheal on skin. Groups of six animals were treated with the test drugs. The drugs were administered intradermal injection.

The percentage of protection was calculated using the formula

$$\text{Percentage protection} = (1 - T_1/T_2) \times 100$$

where T_1 = the mean of occurrence of wheal before administration of test drugs and T_2 = the mean of occurrence of wheal after administration of test drugs at 30 min, 1 h and 4 h. The skin of guinea pig shaved with an electric razor and 0.2% of histamine was injected to the epidermal layer of the skin. Finally noted the time of occurrence of three stages on skin [13].

Antihistamine activity by *in silico* approach

Preparation of protein molecule: The histamine-induced bronchoconstriction was mainly mediated through the histamine H_1 receptor [3RZE] [14]. This G protein coupled the receptor inhibited by H_1 receptor antagonist. Histamine H_1 receptor protein (PDB ID: 3RZE) was retrieved from protein databank PDB (<http://www.pdb.org>). The obtained receptor was energy minimized using SwissPDBViewer [15] after adding the hydrogen bond in Argus Lab. All the water molecules were removed and in the final stage hydrogen atoms were added to the target protein molecule and predict the binding site using Q-Site Finder. It is one of the tools for the binding site prediction [16]. The experiments include preparation of protein molecule, active site prediction, preparation of ligand and molecular docking. Preparation of ligand using bioactive molecules obtained by column chromatography from *T. involucrata* L. leaves was constructed using ChemsSketch 12.0 (www.acdlabs.com) the same way as the standard drugs structures chlorpheniramine and mepyramine were constructed for comparison. 3D structures were geometrically optimized for the ligands according to the Hartree–Fock (HF) calculation method using ArgusLab 4.0.1, generated and optimized to acquire proper geometry, after the active site was found out and the ligand was prepared.

Molecular docking: The molecular docking finds best fit for the bioactive molecules. The interaction can be modeled by a scoring function that includes terms describing the inter- and intra-molecular energies. After storing the selected ligand on .mol file format, docking was made against G-protein coupled receptor (3RZE) and flexible ligand docking was possible, where the ligand is described as a torsion tree and grid. These are constructed using the overlay of the binding site, size of the binding site bounding box was determined automatically using ArgusLab ($34.679000 \times 28.199000 \times 31.398000$ Å). Grid resolution (Ang = 0.400000) and the Lamarckian genetic algorithm were used to perform docking (<http://www.arguslab.com>) and the results were visualized using the PyMOL viewer 1.3.

Statistical analysis

The statistical analysis was performed using Student's "t"-test, one-way analysis-of-variance (ANOVA), followed by Dunnett's test for individual comparison of groups with control.

Results and discussion

Isolation and characterization

The methanol (95%) extract of *T. involucrata* L. leaves concentrated under vacuum to yield 20 g of the residue was subjected to column chromatography. Fractions 18–37 were found to be homogenous by TLC (Table 1) and showed a single spot and the R_f value is 0.37 on concentration yielded at 120.15 ± 1.25 mg of compound 1.

The IR spectrum of compound 1 exhibited a broad absorption band at $3,379\text{ cm}^{-1}$ region, indicating the presence of a –OH group, $1,625\text{ cm}^{-1}$ indicating amide carbonyl group and bands at $1,213\text{ cm}^{-1}$ and $1,045\text{ cm}^{-1}$ indicating the presence of C–N and C–O groups, respectively. The UV-vis spectra showed an absorbance band at 267 nm characteristic of a cyclic amide group. In the NMR spectra (Table 2), the ^{13}C NMR appears to be simple and easy to assign the nature of carbon atoms (Figure 1). So before taking the ^1H NMR the ^{13}C NMR was used to analyze the structure. In the ^{13}C NMR spectrum it exhibited only six signals at $\delta 170.03$, indicating the presence of carbonyl group, at $\delta 71.65$, $\delta 52.39$, $\delta 40.36$ for three carbon atoms attached to hetero atoms like oxygen or nitrogen and the remaining two signals appeared at $\delta 28.39$ and $\delta 22.57$ indicating the presence of two methylene groups. With these signals the tentative structure of the compound was assigned as 5-hydroxy-1-methylpiperidine-2-one; further, it was confirmed by its ^1H NMR. In the ^1H NMR a pair of multiplets at $\delta 1.76$ and $\delta 1.91$ are due to H-4 protons and the multiplets at $\delta 2.28$, $\delta 2.91$ are due to H-3 protons (Figures 2 and 3).

Table 1: TLC profile of *T. involucrata* L.

Eluting solvents	Fractions	TLC solvent systems	No. of spot	Color of spot iodine chamber	R _f value
100% P.E (2)	1–2	100% P.E	–	–	–
90:10	3–8	90:10	2	Pale yellow	0.81
P.E: E.A (13)		P.E: E.A			0.72
	9–13	90:10	3	Pale yellow	0.91
		P.E: E.A		Yellow	0.80
				Green	0.61
80:20	14–28	90:10	3	Pale yellow	0.82
P.E: E.A (72)		P.E: E.A		Yellow	0.76
				Green	0.64
	29–86	90:10	2	Yellow	0.54
		P.E: E.A		Green	0.65
70:30	87–92	80:20	2	Green	0.62
P.E: E.A (6)		P.E: E.A		Dark green	0.57
60:40	93–98	70:30	3	Dark green	0.81
P.E: E.A (5)		P.E:E.A		Green	0.70
					0.62
50:50	99–105	60:40	2	Dark green	0.71
P.E: E.A (6)		P.E:E.A			0.64
40:60	106–119	50:50	2	Dark green	0.81
P.E: E.A (16)		P.E: E.A			0.70
100% E.A (5)	1–5	50:50	2	Pale green	0.70
		P.E: E.A		Green	0.61
100 % Ch (5)	1–5	100% Ch	2	Yellow	0.35
					0.41
80:20	1–5	90:10	2	Yellowish brown (A.I)	0.56
Ch:MeOH (5)		Ch:MeOH			0.45
50:50	6–41	80:20	2	Yellowish brown (A.I)	0.67
Ch:MeOH (37)		Ch:MeOH			0.60
100% MeOH	1–17	50:50	2	Brown (A.I)	0.45
		Ch:MeOH			0.65
	18–37	100% MeOH	1	Dark brown (A.I)	0.37

P.E., petroleum ether; E.A., ethyl acetate; Ch, chloroform; MeOH, methanol; A.I, after iodination.

Table 2: ¹H-NMR and ¹³C NMR signals of compound 1.

Carbon	Signal (δ)	Proton	Signal (δ)
1	–	–	–
2	170.03	H-2	–
3	22.57	H-3	2.28 (1H,m)
4	28.59	H-4	2.91 (1H,m)
5	70.17	H-5	1.76 (1H,m)
6	52.39	H-6	1.91 (1H,m)
7s	40.36	H-7	3.57 (1H,m)

The multiplets at δ3.1 and δ3.5 are assigned to H-6 protons. The proton H-5 under the oxygen function resonated at δ3.57 as a multiplet. The N–CH₃ proton appeared as a singlet at δ2.75. Mass spectrum showed a molecular ion peak at *mz* 129.16, suggesting a molecular formula of C₆H₁₁NO₂. Based on the UV-vis, FT- IR, NMR and MS spectral data, the structure was predicted for the compound 1 as 5-hydroxy-

1-methylpiperidine-2-one (Figure 4). This is piperidine-based alkaloid like phenylmethyl piperidine derivatives; it already reported against allergic disease [17].

Antihistamine activity of 5-hydroxy-1-methylpiperidin-2-one

Isolated guinea pig ileum preparation

The syndrome of bronchial asthma is characterized by widespread narrowing of the bronchial tree due to contraction of the smooth muscle in response to multiple stimuli, resulting in the release of chemical mediator such as histamine. Guinea pig ileum is used for screening of antihistamine in response to anti-asthmatic activity. The stimulation of H₁ receptors produces graded dose-related contraction of isolated guinea pig ileum [18].

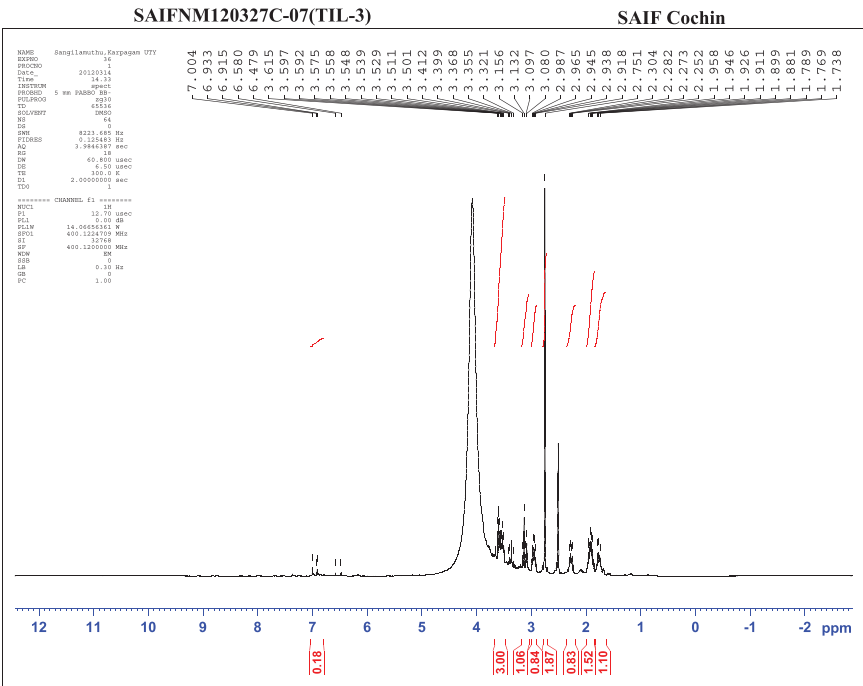


Figure 1: ¹H NMR spectra of compound 1.

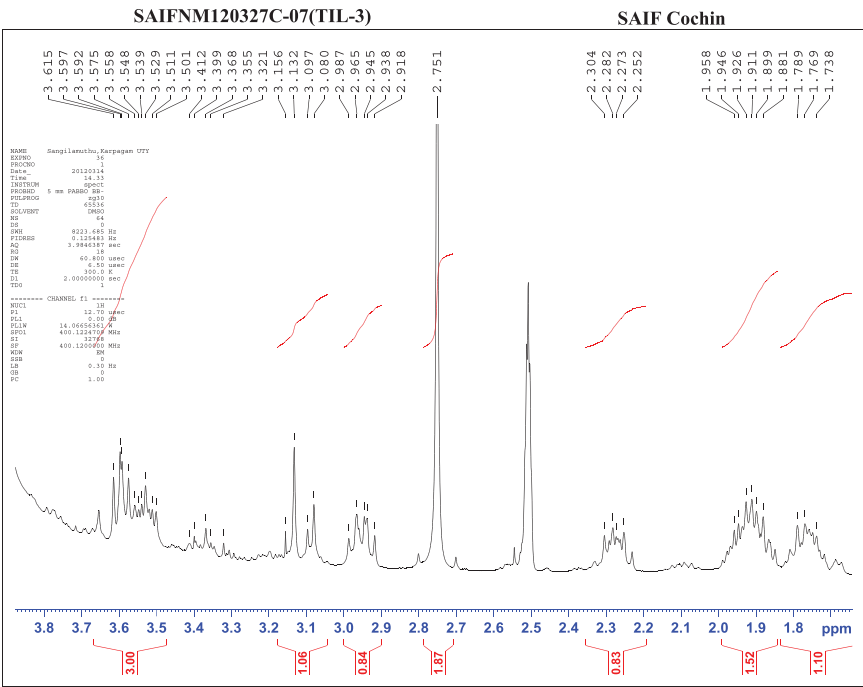


Figure 2: ¹H NMR spectra of compound 1 with expansion.

The muscle contraction effect of 5-hydroxy-1-methylpiperidin-2-one (12.5 µg/mL) in different dose levels by histamine induced muscle contraction (35.17 ± 1.13 to $97.16 \pm 2.25\%$ in control; 17.18 ± 1.15 to $52.15 \pm 2.25\%$ in compound 1; 20.10 ± 1.55 to $55.25 \pm 1.65\%$ in CPM treated) on guinea pig

ileum preparation (Table 3) and the results show that 5-hydroxy-1-methylpiperidin-2-one possesses muscle relaxant effect compared with CPM. The different doses of histamine (10 µg/mL), compound 1 (12.5 µg/mL) and CPM (10 µg/mL) between 0.1 and 3.2 mL were screened and

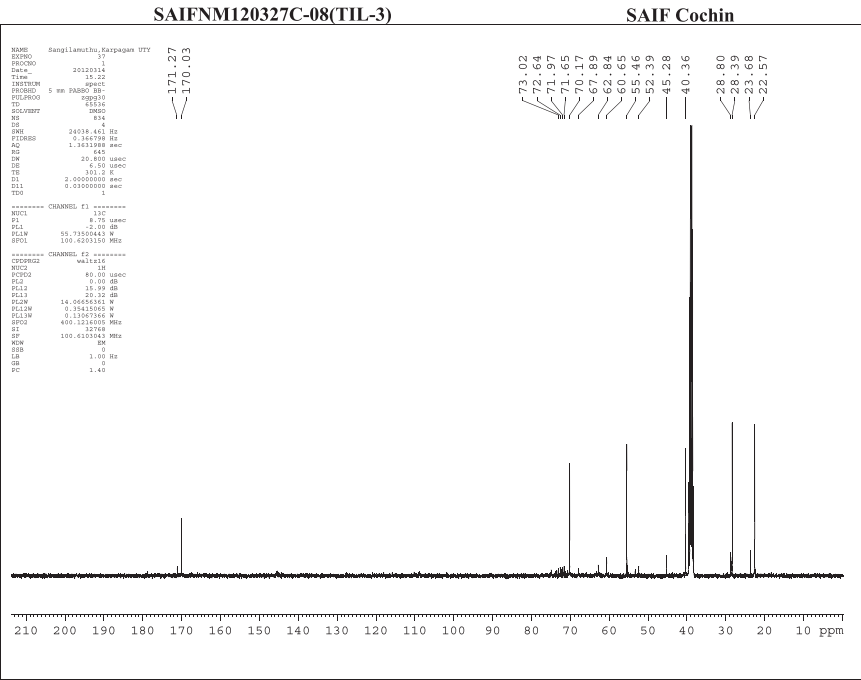
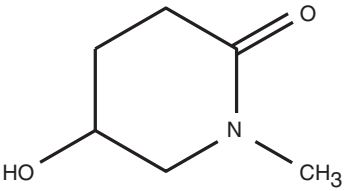


Figure 3: ¹³C NMR spectra of compound 1.



5-Hydroxy-1-methylpiperidin-2-one

Figure 4: Chemical structure of compound 1.

Table 3: Effect of 5-hydroxy-1-methylpiperidin-2-one on histamine-induced contraction in isolated guinea pig ileum.

Dose, mL	Control (10 µg/mL), %	Compound 1 (12.5 µg/mL), %	CPM (10 µg/mL), %
0.1	35.17 ± 1.13	17.18 ± 1.15	20.10 ± 1.55
0.2	55.25 ± 2.15	29.15 ± 1.55	31.25 ± 1.25
0.4	62.35 ± 1.17	31.45 ± 2.01	35.75 ± 2.01
0.8	71.20 ± 2.05	36.66 ± 1.35	36.55 ± 1.55
1.6	85.45 ± 2.50	42.55 ± 1.15	45.25 ± 2.25
3.2	97.16 ± 2.25	52.15 ± 2.25	55.25 ± 1.65

Control (10 µg/mL): DRC of histamine in the absence of 5-hydroxy-1-methylpiperidin-2-one (compound 1); compound 1 (12.5 µg/mL): DRC of histamine in the presence of 5-hydroxy-1-methylpiperidin-2-one (compound 1); CPM (10 µg/mL): DRC of histamine in the presence of chlorpheniramine maleate which is standard.

found to be the significant muscle relaxant effect. The results show the percentage of contraction reduced in compound 1 and CPM treated tissue compared to the control; it shows that our test drug possesses muscle relaxant effect.

Histamine-induced bronchoconstriction in guinea pigs

Histamine inhalation causes hypoxia and leads to convulsion in guinea pigs and causes very strong smooth muscle contraction, profound hypotension and capillary dilation in cardiovascular system. A prominent effect caused by histamine leads to severe bronchoconstriction in the guinea pigs that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms [19]. Histamine-induced bronchoconstriction was screened using isolated 5-hydroxy-1-methylpiperidin-2-one with different concentrations of 7.5, 10.0 and 12.5 mg/kg and found to be $38.10 \pm 1.90\%$, $51.14 \pm 2.56\%$ and $55.54 \pm 2.78\%$ protection against histamine-induced bronchoconstriction, respectively, and also latent period of convulsant was increased up to 18.33 ± 1.32 min at the dose level of 12.5 mg/kg p.o. compared to CPM. From this calculated the IC_{50} value of 5-hydroxy-1-methylpiperidin-2-one and found 9.75 mg/kg. It shows that the isolated 5-hydroxy-1-methylpiperidin-2-one possesses antihistamine activity followed by bronchodilating effects (Tables 4 and 5). It indicates that the isolated 5-hydroxy-1-methylpiperidin-2-one acts as a potent antihistamine agent. Drug's effectiveness to asthma is mostly alkaloid and steroidal in nature.

Bronchial asthma is commonly characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of

Table 4: Effect of 5-hydroxy-1-methylpiperidin-2-one on histamine-induced bronchoconstriction.

Groups	Latent period of convulsion, min (mean $\pm$ SEM)			
	Before	30 min	1 h	4 h
IC (–ve control)	7.09 $\pm$ 0.35 ^a	5.10 $\pm$ 0.25 ^a	6.22 $\pm$ 0.30 ^a	4.35 $\pm$ 0.22 ^a
STD (+ve control)	7.31 $\pm$ 0.36 ^b	14.27 $\pm$ 1.10 ^b	21.37 $\pm$ 1.06 ^b	12.27 $\pm$ 1.00 ^b
5-Hydroxy-1-methylpiperidin-2-one (7.5 mg/kg)	7.15 $\pm$ 0.25 ^a	9.25 $\pm$ 1.05 ^c	11.55 $\pm$ 1.30 ^c	8.25 $\pm$ 1.05 ^a
5-Hydroxy-1-methylpiperidin-2-one (10 mg/kg)	7.49 $\pm$ 0.37 ^c	11.46 $\pm$ 0.57 ^c	15.33 $\pm$ 1.16 ^c	10.13 $\pm$ 0.50 ^c
5-Hydroxy-1-methylpiperidin-2-one (12.5 mg/kg)	8.15 $\pm$ 0.40 ^d	14.17 $\pm$ 1.10 ^b	18.33 $\pm$ 1.32 ^d	12.21 $\pm$ 1.00 ^b

Values are expressed as mean $\pm$ SD for five animals in each group. Values not sharing common superscript letters (a–d) differ significantly at $p < 0.05$ (DMRT).

Table 5: Percent protection of 5-hydroxy-1-methylpiperidin-2-one against histamine-induced bronchoconstriction in guinea pig.

Groups	% Protection		
	30 min	1 h	4 h
STD; Chlorpheniramine maleate (2 mg/kg, i.p.).	48.77 $\pm$ 2.44	65.79 $\pm$ 2.25	59.58 $\pm$ 2.98
5-hydroxy-1-methylpiperidin-2-one (7.5 mg/kg)	22.70 $\pm$ 1.14	38.10 $\pm$ 1.90	13.33 $\pm$ 0.67
5-hydroxy-1-methylpiperidin-2-one (10 mg/kg)	34.64 $\pm$ 1.73	51.14 $\pm$ 2.56	26.06 $\pm$ 1.30
5-hydroxy-1-methylpiperidin-2-one (12.5 mg/kg)	42.48 $\pm$ 2.12	55.54 $\pm$ 2.78	33.25 $\pm$ 1.66

Table 6: Effect of 5-hydroxy-1-methylpiperidin-2-one on histamine induced triple response in guinea pigs.

Groups	Latent period of wheal, min (mean $\pm$ SEM)			
	Before	30 min	1 h	4 h
IC (–ve control)	7.25 $\pm$ 0.37 ^a	5.18 $\pm$ 0.26 ^a	8.13 $\pm$ 0.41 ^a	6.22 $\pm$ 0.31 ^a
STD (+ve control)	7.44 $\pm$ 0.38 ^b	11.2 $\pm$ 0.55 ^b	15.14 $\pm$ 1.16 ^b	13.11 $\pm$ 1.05 ^b
5-Hydroxy-1-methylpiperidin-2-one (10 mg/kg)	6.31 $\pm$ 0.32 ^c	7.10 $\pm$ 0.36 ^b	9.12 $\pm$ 0.45 ^b	8.26 $\pm$ 0.41 ^b
5-Hydroxy-1-methylpiperidin-2-one (12.5 mg/kg)	7.27 $\pm$ 0.46 ^a	9.32 $\pm$ 0.46 ^d	14.27 $\pm$ 1.11 ^d	11.28 $\pm$ 0.57 ^d

Values are expressed as mean $\pm$ SD for five animals in each group. Values not sharing common superscript letters (a–d) differ significantly at $p < 0.05$ (DMRT).

inflammatory mediators like histamine triggered by exposure to allergens that directly cause acute bronchoconstriction [20]. In this study we developed a guinea pig model through inhalation of histamine aerosol by pre- and posttreatment of 5-hydroxy-1-methylpiperidin-2-one as antihistamine drug to prevent sudden breathing difficulties due to systematic hypersensitivity toward anti-asthmatic effect.

Histamine induced triple response in guinea pig

The histamine induced triple response on guinea pig, their skin was seen as red spot, flare and wheal at different time intervals and recorded the time duration to occur these reactions on skin (Table 6). Histamine was injected intradermally, after getting reaction

(red spot, flare and wheal) on skin the test drug (5-hydroxy-1-methyl piperidin-2-one) and standard drug were injected by same way and time of disappearance of flare and wheal in skin were noted (Figure 5), calculated the percentage protection of test drug. The percentage protection of 5-hydroxy-1-methylpiperidin-2-one (12.5 mg/mL) was found to be $49.05 \pm 2.26\%$ compared to the CPM ($50.86 \pm 2.54\%$). This shows that the test drug possesses anti-allergic activity toward the antihistamine activity (Table 7).

The reaction of wheal occurrence on the skin started at 30 min, then recorded the same time up to 4 h on the injected site of the skin. CPM (2 mg/kg, i.p.) reduced the formation exudation fluid showing as flare and wheal in Figure 2. Thirty minutes after the test drug was injected and it inhibited the histamine response to about 30% and 45%. The inhibitory effect on histamine-induced guinea pig

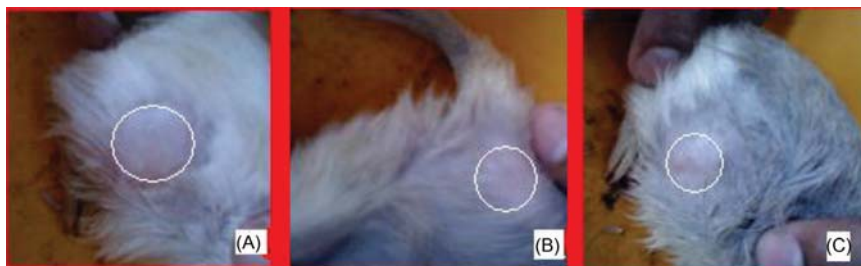


Figure 5: Effect of 5-hydroxy-1-methylpiperidin-2-one against histamine-induced triple response on skin. (A) Control (–ve); (B) CPM-treated animal; and (C) test drug-treated animal.

Table 7: Percent protection of 5-hydroxy-1-methylpiperidin-2-one against histamine-induced triple response.

Groups	% Protection		
	30 min	1 h	4 h
STD	33.57 ± 1.68	50.86 ± 2.54	43.25 ± 2.16
5-Hydroxy-1-methylpiperidine-2-one (10 mg/kg)	11.13 ± 0.56	30.81 ± 1.54	23.61 ± 1.18
5-Hydroxy-1-methylpiperidin-2-one (12.5 mg/kg)	22.00 ± 1.10	49.05 ± 2.45	35.55 ± 1.78

STD, chlorpheniramine maleate (2 mg/kg, i.p.).

was prevented by CPM (2 mg/kg, i.p.) as histamine H_1 receptor antagonist. The isolated 5-hydroxy-1-methylpiperidin-2-one significantly influences the antigen response.

Antihistamine activity of 5-hydroxy-1-methylpiperidin-2-one against histamine H_1 receptor by in silico

In vitro and *in vivo* antihistamine activity of 5-hydroxy-1-methylpiperidin-2-one was found to be significant and this was confirmed by *in silico* approach compared to the standard antihistamine drugs CPM and mepyramine. The test drug and standard drugs' chemical structures were drawn by ChemDraw software and saved on .mol file format as ligand. Histamine H_1 receptors (3RZE) retrieved from PDB, as per "Materials and methods" section, were docked using Argus Lab and give the score value –9.76, –10.61 and –9.76 for 5-hydroxy-1-methylpiperidin-2-one, chlorpheniramine and mepyramine, respectively (Table 8); these score values show that the 5-hydroxy-1-methylpiperidin-2-one contains good inhibition against histamine H_1 receptor due to the hydrogen bond interaction of the ligand (Figure 6).

Piperidine-based alkaloids are well-known histamine antagonists. The major piperidine-based alkaloids are terfenadine [21], azatadine, fexofenadine [22], loratadine [23], cyproheptadine, ketotifen [24], chlorpheniramine [25] and emedastine [26] reported against histamine H_1 and H_3 receptors or as antihistamine drugs. Most of the histamine antagonist drugs contain

Table 8: Best score and energy value of 5-hydroxy-1-methylpiperidin-2-one against histamine H_1 receptor.

S. No.	Compound name	Docking score, kcal/mol
1	5-Hydroxy-1-methylpiperidin-2-one	–9.76
2	Chlorpheniramine (STD drug)	–10.61
3	Mepyramine	–9.76

piperidine ring structure, the isolated compound also contain such ring along with CH_3 group and = O group attached in the ring structure.

Conclusions

In conclusion, the isolated 5-hydroxy-1-methylpiperidin-2-one from methanol extract of *T. involucrata* L. leaves potent bioactive molecule for muscle relaxant, broncho-dilating and anti-allergic effects on histamine induced muscle contraction in ileum, bronchoconstriction in bronchioles and triple response in the skin of guinea pig. These activities were confirmed by molecular docking against histamine H_1 receptor (3RZE). 5-Hydroxy-1-methylpiperidin-2-one showed highest docking score, energy and interactions compared with the CPM and mepyramine. The 5-hydroxy-1-methylpiperidin-2-one and its mechanism of action explained for the observed

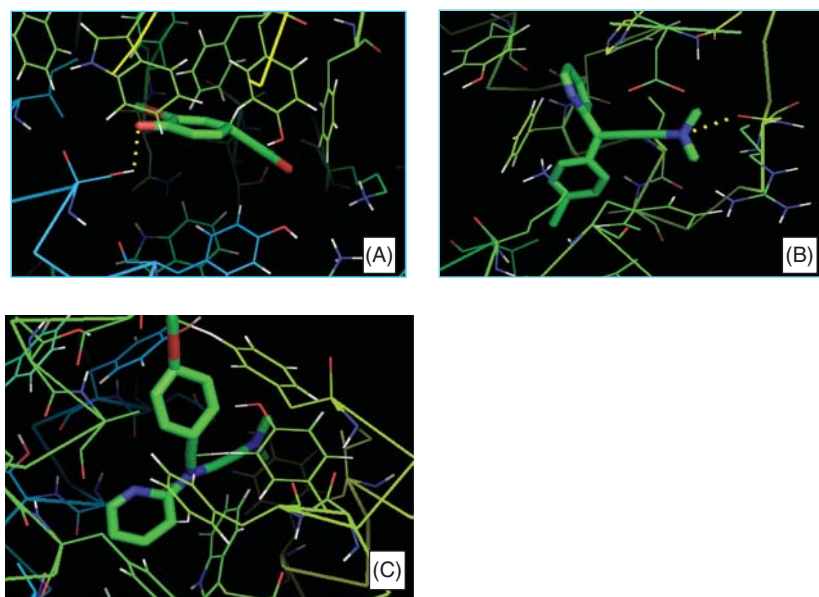


Figure 6: Molecular interactions against histamine H1 receptor. (A) 5-Hydroxy-1-methylpiperidin-2-one; (B) chlorpheniramine; and (C) mepyramine.

activities have not been established with clinical trials and thus further investigation needs to be conducted.

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