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Research Article

Evaluation of anti-histaminic activity ofaqueous extract of *ripe olives of olea-europea*

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ABSTRACT

According to Ayurveda, *Olea europea* is used in treatment of leprosy, dysentery, vaginal and uterine complaints, inflammation, burning sensation, fatigue, asthma, leucoderma etc. In the present study the histamine induced dose dependent contraction of goat tracheal chain and guinea pig ileum preparation was significantly inhibited ($p< 0.01$) by the aqueous extract of leaves of *Olea europea* (800 ug/ml and 100 ug/ml). *Olea europea* at the doses of 100and 200 mg/kg,i.p.,exhibited significant ($p< 0.01$) mast cell stabilizing activity against clonidine induced peritoneal mast cell degranulation in rats. Prior treatment with *Olea europea* extract (6.6 mg/kg and 10.2 mg/kg, i.p) significantly inhibited the clonidine-induced catalepsy in mice but, did not show any significant decrease in haloperidol induced catalepsy. Thus the present study revealed that the extract of olives of *Olea europea* has significant antihistaminic (H1 receptor antagonist) activity.

INTRODUCTION

Alternative system of medicine viz. Ayurveda, siddha, and Chinese medicine have become more popular in recent years. *Olea europea* belonging to the family oleaceae is a small evergreen tree, from 12 to 20 feet high, with hoary, rigid branches, and a grayish bark[1] In many countries extract of *olea europea* is used in the treatment of headache,migraine,insomnia,diarrhea,dysentery,fever, pile and fistula. The phytochemical characteristics show the presence of tannins,steroids,alkaloids, triterpens and flavonoids.[1], etc. These exert physiological and therapeutic effect. *Olea europea linn* is commonly found plant in moist waste ground, lawns, open plantation and weedy thickets[24,25]. It is native of middle America and now widely distributed in all tropical areas[1]. The leaves are opposite,lanceolate,or ovate-lanceolate,mucronate, short-petiole, green above, and hoary on the underside. The flowers are small,in short, axillary, erect racemes, very much shorter than the leaves[4] Most of plant parts of *olea europea* are used in traditional system of medicine in world. Oil is taken with lemon juice to treat gallstones[26]. Decoctions of the dried fruit and of dried leaf are taken orally for diarrhea and to treat respiratory and urinary tract infections. Seed oil is taken orally as a laxative and applied externally as an emollient and pectoral.. Hot water extract of dried plant is taken orally for bronchial asthma. Infusion of the fresh leaf is taken orally for as an anti-inflammatory[28]. An olive leaf extract was reported in a laboratory study to have vasodilating effects, seemingly independent of vascular endothelial inregrity. Traditional uses support olive leaf and olive oil in cardiovascular disease prevention. Additionally, olive leaf extracts may possess antispasmodic, vasodilator, and anti-arrythmic properties.

According to Ayurveda, *Olea europea* is used in treatment of biliousness, leprosy, dysentery, vaginal and uterine complaints,inflammations,burning sensations, fatigue, asthama,leucoderma and blood diseases. The aqueous extract of Olea europea produced an antidepressant like profile similar to tricyclic antidepressant. The decoction of *Olea europea* shows anticonvulsant and hyperglycemic activity. Histamine is an autocoid having profound physiological effect in the body and is one of the important mediators of allergy, inflammation and bronchoconstrictionS. Targeting histamine, either prevention of its release from mast cells or use of histaminergic receptor antagonists become part of antihistaminic therapy in allergic inflammatory diseases[7].

The present study was planned to evaluate the antihistaminic action of aqueous extract of *olea europea* using various models like, goat tracheal chain preparation, isolated guinea pig ileum, clonidine induced mast cell degranulation, and clonidine and haloperidol induced catalepsy in mice.

MATERIALS AND METHODS

Plant material and drugs

The *Olea europea* were collected from local nursery of India and agricultural area around Pune city region. The plant specimen was authenticated . The drug were purchase from sources

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indicated in parenthesis

Preparation of extract of *Olea europea* for the biological tests

The *Olea europea* were air dried. After 10 days of drying, the olives were coarsely powdered using a mixer. The extract was prepared by a percolation method. The solvent used for the extraction was distilled water. The extract was concentrated and then dried at 40 and percent yield obtained was 25% w/w[8].

Animals

All experimental procedure was carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and Supervision on Experimentation on Animals (CPCSEA)and approved by the institutional Animal Ethics Committee. Goat trachea was obtained from the slaughterhouse and immersed in Kreb solution at 37^0c. Albino swiss mice weighing between 20 to 25 gm and Dunkin Hatley guinea pigs weighing between 350 to 400 gm were used. The above animals of either sex were purchased from National Toxicological Center,Pune. They were housed in groups of three under standard laboratory condition of temperature (23± 2) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized,throughout the experiment.

Dose selection

The extract of olives of *Olea europea* has been reported to possess antidepressant activity at various doses that is on 2,6 and 8 mg/kg, intraperitoneally[5]. From this we select the dose that is 6 and 8 mg/kg in rats for the experimental model. While in case of mice the corresponding dose were calculated using conversion factor[9]. The dose 500 ug/ml for guinea pig ileum preparation were selected by our laboratory trials.

Goat tracheal chain preparation

Goat trachea was obtained immediately after slaughter of the animals. Tracheas was cut into individual rings and tied together in series to form a chain and suspended in bath containing Krebs solution at 37±0.5 and allow to equilibrate for 45 minute under a load of 400 mg. A dose response curve for histamine was recorded at variant molar concentrations, by maintaining 15 min time cycle[10,11]. After obtaining a dose response curve of histamine on trachea, the *Olea europea* extract was added to the reserviour and same doses of histamine were repeated. Graph of percentage of maximum contractile responses on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence Olea euripea extract.

Guinea pig ileum preparation

Overnight fasted guinea pig was sacrificed and ileum mounted in an organ bath containing Tyrode solution, which was continuously aerated and maintained at temperature 37±0.5. The tissue was allowed to equilibrate for 30 minute under a load of 500 mg, contact time of 30 seconds and 5 minute time cycle was followed for recording the response curve of histamine (10ug/ml) on ileum[12], the Olea europea extract (100ug/ml) was added to the reserviour and same doses of histamine were repeated in presence of *Olea europea* extract.

Clonidine-induced peritoneal mast cell degranulation in rat

Rats were divided in four groups and a seven day drug treatment schedule was followed, Group I received only the distilled water (10ml/kg, i.p). Group II was treated with sodium cromoglycate (50mg/kg,i.p.). Groups III and IV were treated with aqueous extract of Olea europea 4 and 8 mg/kg,i.p, respectively. On the 7^{th} day, two hours after the assigned treatment mast cells were collected from the peritoneal cavity. The rats were anesthetized with ether and were injected 10 ml of normal saline solution into the peritoneal cavity and the abdomen was gently massaged for 90 seconds. The peritoneal cavity was carefully opened and the fluid containing mast cell was aspirated and collected in siliconised test tube containing 7 to 10 ml of RPMI-1640 Medium (PH 7.2-7.4)

The mast cells were then washed three times by centrifugation at low speed (400-500 rpm) and the pallet of mast cells was taken in the RPMI-1640 medium (PH 7.2-7.4)

Then 0.5 mcg/ml of clonidine solution was added to the mast cell suspension (approxiamately 1/10 cells/ml) and incubated at 37 in a water bath for 10 min. Late they were stained with 1% toludine blue and observed under light power microscope field (400 X). A total of 100 cells were counted from different visual areas and percent protection against clonidine induced mast cell degranulation was calculated[13,14].

Clonidine induced catalepsy in mice

Bar-test used to study the effect of test drugs on clonidine-induced catalepsy. Mice were divided into four groups . Animals belonging to group I served as control and were administered the vehicle (distilled water 10ml/kg,i.p). Animals belonging to group II received chlorpheniramine maleate (10 mg/kg, i.p) where as groups III and IV received Olea europea in doses (5.6 and11.2 mg/kg,i.p) respectively. The forepaws of mice were placed on a horizontal bar (1 cm in diameter,3 cm above the table) and the time required to remove the paws from bar was noted for each animal. All the groups received clonidine (1 mg/kg,s.c), 1 hr after the drug administration and duration of catalepsy was noted at 15,30,60,90,120,150

and 180 minute time intervals.

Haloperidol-induced catalepsy in mice[15,16]

The same Bar-test was used to evaluate the effect of test drugs on the haloperidol induced catalepsy in mice, instead of clonidine here haloperidol (1 mg/kg,i.p) was administered one hour after assigned treatment. The duration of catalepsy was noted at 15,30,60,90,120,150, and 180 minute time intervals.

Statistical Analysis

All observations were presented as mean + SEM. The data analyzed by students t-test or one way ANOVA followed by Dunnets test. Values of P< 0.05 were considered as significant result.

RESULTS

Goat Tracheal chain preparation

In the present study, histamine (30ug/ml) produced dose dependent contraction of goat tracheal chain preparation as indicated in the graph of maximum percentage of contractile response v/s negative log molar concentration of histamine.The modified physiological salt solution containing aqueous extract of Olea europea (500 ug/ml) significantly inhibited ($p<0.01$) the contractile effect of histamine.

Guinea pig ileum preparation

In the present study, histamine produced dose dependent contraction of guinea pig ileum preparation as indicated in the graph of maximum percentage of contractile response v/s negative log molar concentration of histamine. The modified physiological salt solution containing the extract of Olea europea (200 ug/ml) significantly inhibited ($p<0.001$) the contractile effect of histamine on isolated guinea pig ileum preparation.

Clonidine induced peritoneal mast cell degranulation in rats

Clonidine challenge resulted in significant peritoneal mast cell degranulation in rat. Pretreatment of animal with extract of Olea europea (6 mg/kg, and 8mg/kg,i.p) resulted in significant reduction ($p<0.01$) in degranulation of mast cell when challenged with clonidine and the percent protection was found to be 50.82 and 65.11% respectively. Sodium cromoglycate (50mg/kg,i.p) significantly inhibited ($p<0.01$) clonidine induced mast cell degranulation and percent protection was found to be 71.42 .

Table 1- Effect of *Olea europea* on clonidine-induced mast cell degranulation in rats

Group	Treatment	Mast cells %		Percent protection
I	Control	17.4± 0.93	82.6± 1.92	——
II	Sodium cromoglycate	76.4± 1.50	3.61±.85	71.42
III	*Olea europea* (6 mg/kg, i.p.)	61.0± 1.85	46.0± 1.85	50.82
IV	*Olea europea* (8 mg/kg, i.p)	71.0± 1.72	35.0± 1.72	65.11

Clonidine induced catalepsy in mice

Clonidine (1mg/kg,s.c) produced catalepsy in mice, which remained for 3 hours. The vehicle treated group has shown maximum duration of catalepsy at 120 minute after the administration of clonidine. There was significant inhibition ($p<0.05$ and $p<0.01$) of clonidine induced catalepsy in the animals pretreated with Olea europea extract at dose (5.6 and 11.2 mg/kg,i.p). Chlorpheniramine maleate (10 mg/kg.i.p.) treated group significantly reversed ($p<0.01$) clonidine-induced catalepsy in mice.

Table 2- Effect of *Olea europea* on clonidine-induced catalepsy in mice

Group	15 min	30 min	60 min	90 min	120 min	150 min	180 min
I	25±1.36	100±1.49	240±3.42	250±1.99	290±2.79	260±2.80	200±3.69
II	15±1.86	20±1.39	21±2.50	54±2.48	78±2.55	115±1.36	125±2.22
III	23.6±1.36	106.2±2.45	230.4±2.61	236.6±3.09	239.6±1.74	250.2±2.85	193.8±2.69
IV	16.0±2.31	63.0±2.85	141±3.29	150±2.67	180±3.74	190±2.08	182±3.49

Haloperidol-induced catalepsy in mice

Mice pretrated with extract of Olea europea at doses of 6.6 and 10.2 mg/kg,i.p did not show any significant inhibition of haloperidol induced catalepsy.Chlorpheniramine maleate (10 mg/kg,i.p) treated group also failed to reverse haloperidol-induced catalepsy in mice.

Table 3- Effect of *Olea europea* on Haloperidol-induced catalepsy in mice

Group	15 min	30 min	60 min	90 min	120 min	150 min	180 min
I	90±7.0	130±11.0	250±9.81	200± 8.11	211.7±4.82	179.2±9.30	168.2± 8.80
II	4.6±10.50	159.6±9.02	269.8±10.51	253.2±8.02	212.5±9.54	188.7±7.67	153.4±10.18
III	87.7±13.03	132.2±10.06	250.6±5.28	200.9±7.39	200.7±6.01	190.78±7.84	110.5±9.05
IV	95.8±7.12	148.8±3.03	273.4±6.45	247.5±6.30	214.2±7.60	182.5±7.31	160.5±7.23

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DISCUSSION

Histamine is one of the important mediators of allergy, inflammation, and bronchoconstriction which was released after degranulation of mast cell by an antigenic exposure.Targeting histamine, either prevention of its release from mast cells or use of histaminergic receptor antagonists becomes part of antihistaminic therapy in allergic diseases[7].

The contraction of tracheal or bronchial smooth muscle in vitro has often been utilized for the study of contractile /dilator responses of agonists as well as antagonist. Both goat tracheal chain and strip preparation are suitable for screening the activity of a drug on respiratory smooth muscles.[11] Spasmogens such as histamine, acetylcholine and barium chloride produced dose dependent contraction of goat tracheal chain preparation. The goat tracheal muscle has histamine H1,M3.B2 receptors . The stimulation of histamine H1 receptors cause contraction of bronchiole smooth muscles. In present study, Olea europea extract (500 ug/ml) significantly inhibited histamine induced contraction of goat tracheal chain preparation, indicating histamine H1 receptor antagonist activity. Guinea pig ileum is use for serrening of anti-histaminic activit. The stimulation of H1 receptor produces graded dose related contraction of isolated guinea pig ileum[10]. In the present study, *Olea europea* (500 ug/ml) in tyrode solution significantly inhibited histamine induced contraction of guinea pig ileum preparations indicating H1 receptors antagonistic activity. Mast cells are widely distributed in the connective tissue, with preferential localization adjacent to small blood vessel. Sodium cromoglycate standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate.The pharmacological agent that increase intracellular level of cAMP relaxes smooth muscles and inhibit the release of Autacoids and tissue and basophils.

The groups of animals pretreated with aqueous extract of Olea europea (6 mg/kg, 8mg/kg, i.p) resulted in significant reduction ($p<0.01$) in degranulation of mast cells and offered 50.82 and 65.11 percentage protection respectively when challenged with clonidine.Clonidine, a a-2 adreno receptor agonist induces dose dependent catalepsy in mice, which is inhibited by histamine H1 receptor antagonist but not by H2 receptor antagonist[19]. The intracerebro-ventricular (i.c.v) injection of histamine in conscious mice induce catalepsy which was inhibited by histamine H1 rceptor but not by H2 receptor antagonist[20]. Clonidine causes degranulation of mast cells like compound 48/80 and release of inflammatory mediators such as histamine[21], Leukotrines, protaglandines etc. In the present study, prior treatment with *Olea europea* extract significantly inhibited the clonidine induce catalepsy in mice. The effect of Olea europea on clonidine induced catalepsy may be due its anti-histaminic (H1receptor antagonist activity)

The haloperidol induces catalepsy by inhibiting the dopamine D2 receptor in substantia nigra[22,23]. The present study demonstrated that there was no inhibition of haloperidol induce catalepsy in the groups pretrated with *Olea europea* chlorphinarimne maleate (H1 receptor antagonist). Thus it can be concluded that aqueous extract of ripe olives of Olea europea possess significant anti-histaminic (H1 receptor antagonist activity.). aqueous extract of ripe olives of *Olea europea*, virtue of its anti-histaminic activity can be used in the treatment of allergic diseases Hence further study needs to identification, isolation, purification and evaluation of active constituent present in the *Olea europea* ripe olives for its anti-histaminic activity.

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