Evaluation of Anti Histaminic Activity of Hydro Alcoholic Extract of Prunus Domestica Fruits in Guinea Pigs

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Abstract - Objective: The aim of this study was to investigate the anti histaminic activity of hydroalcoholic extract of prunus domestica fruits in guinea pigs Method:The antihistaminic activity is evaluated by the isolated guinea pig ileum method and histamine induced broncho constriction method. Results: The result showed that 400mg/kg of prunus domestica hydroalcoholic extract showed significant anti histaminic activity when compared to the 200mg/kg in histamine induced broncho constriction method compared to the isolated guinea pig ileum method and Chlorpheniramine maleate was used as a standard drug. Conclusion: Hydroalcoholic extract of prunus domestica fruit showed significant protected the guinea pigs against histamine induced bronchospasm.

Keywords: Prunus domestica, Broncho constriction, Histamine, Guinea pig.

I. INTRODUCTION

Prunus domestica is an edible plant with fruits that have been used in India, China and Thailand for used as a seasonal edible fruit, since it has anti-bacterial activity[1],anti oxidant activity[2], anti rheumatic activity, memory enhancer3,. Hydro alcoholic extract of P. domestica have also been shown the hepato protective activity[4].P. Domestica is a member of the rosaceae family and its have a sweet aromatic flavour . Mast cells or use of histaminergic receptors antagonists becomes part of antihistaminic therapy. In human body histamine was present in various biological fluids, in platelets, leucocytes, basophiles and mast cells. Major portion of histamine was stored in mast cells and circulatory basophiles Histamine also acts as a neurotransmitter participating in many cell physiological processes such as allergic reaction, inflammation, and gastric acid secretion, central and peripheral neurotransmission

MATERIALS AND METHODS:

A. Plant Materials Procurement and Authentication

Procurement of plant materials of the fruits of P.domestica were collected from local habitat Mangalagiri, Dist-guntur, (Andhra pradesh) India. Authentication of Plant for the present study was authenticated by Dr. P. Satyanarayana Raju, Department of Botany, Acharya Nagarjuna University, Guntur.

Fig. 1.1 Fruit of prunus domestica

B. Preparation of Extract

The fruits of prunus domestica was taken and peeled off and it were cut into small pieces.. The pieces of leaves were placed in a round bottom flask with distilled water and alcohol. The hydro alcoholic distillation was carried out for 4 h.

C. Phytochemical Screening of Plant Extract

The main chemical components in the extracts were characterized by coloured reactions, alkaloids (reagents of Dra-gendroff and Mayer), the flavanoids (reaction of cyanidine), the Glycosides5,6 (Borntragers test). Results of preliminary phytochemical screening of hydro alcoholic extract of prunus domestica fruits are shown in Table No:1

D. Experimental Animals

Dunkin-Hartley Guinea pigs[7] weighing 350 to 400 g were used for the present study. Animals were housed under a standard 12 h: 12 h light/dark cycle and were provided with food and water ad libitium. Animals were acclimated to laboratory conditions before testing. Each animal was used once. The animal protocol was approved by the Institutional Animal Ethical Committee and the study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

E. Acute toxicity studies according to O.E.C.D. guidelines

Acute toxicity studies according to O.E.C.D. guideline acute toxicity studies on the fruit extract of prunus domestica was calculated. LD50 of test compounds were performed at Nirmala College of Pharmacy, Mangalagiri, Andhrapradesh (India) as per the O.E.C.D guideline 423. Acute Toxicity Studies are shown in Table No:2Test compounds were suspended in extract solution. The compounds were administered orally to groups of 6 animals. After administration of test compounds the guinea pig were observed for gross behavioral neurological autonomic and toxic effects. The toxicological effects were observed in terms of mortality. No death occurred within 24 h of dose of 100 and 200 mg/kg but at a dose more than 300 mg/kg, 50% mortality was observed. As dose was increased further up to 400 mg/kg, total mortality was found. Hence 200 mg/kg dose was considered as effective dose.

F. Preparation of drug solution

From this acute toxicity studies doses are selected as 200 mg and 400 mg in histamine induced bronchoconstriction. And isolated guinea pig ileum preparation was selected as 100 mg. *P. domestica* hydro alcoholic extract was dissolved in distilled water. Chlorpheniramine maleate was dissolved in distilled water. Histamine was dissolved in physiological saline. Physiological saline was widely recommended as it is known to be compatible with human tissue, and isotonicity with body fluid.

II. EXPERIMENTAL METHODS

A. Histamine Induced Bronchoconstriction in Guinea Pig

Procedure: Overnight fasted guinea pigs were divided into four groups each containing 6 animals[8]. Group 1 was treated as control; Group 2 received standard drug Chlorpheniramine maleate9 (2 mg/kg). Animals belonging to groups 3, 4 received hydro alcoholic extract of prunus domestica (200mg/kg, 400mg/kg). All the doses were given orally. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2% histamine8 aerosol. The preconvulsive time (PCT) was determined from the time of exposure to onset of convulsions . As soon as the PCT were noted, the animal were removed from the chamber and placed in air. 24 h later the animals of Groups 3 and 4 received hydro alcoholic extract of prunus domestica. Group 2 received Chlorpheniramine maleate. These animals were again subjected to histamine aerosol after 1 h of drug administration and PCT was determined. Contractions of Ileum of Pig are shown Figure No:4

The protection offered by treatment was calculated by using the following formula:

Percentage protection = (1 - T1/T2) × 100

Where; T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs.

Values of Histamine Induced Broncho Constriction Method are shown in Table No:3

B. Isolated Guinea Pig Ileum Preparation

Procedure-Overnight fasted guinea pig was sacrificed and ileum was mounted in an organ bath containing Tyrode solution. The composition of Tyrode solution[7] (NaCl 8.0, KCl 0.2, CaCl2 0.2, MgCl2 0.1, NaHCO3 1.0, NaH2PO4 0.05 and glucose 1.0 g/L) which was continuously aerated and maintained at 37 ± 0.5ºC. One end of ileum was attached to an S-shaped aerator tube and other attached to isotonic frontal writing lever to smoked drum. The tissue was allowed to equilibrate for 30 min. under a load of 500 mg. Contact time of 30 s and 15 min time cycle was followed for recording the response of Histamine. After obtaining a dose response curve of histamine on ileum, the P.domestica hydro alcoholic extract (100 µg/ml) was added to the reservoir and same doses of histamine were repeated in presences of extracts. Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of extracts.Values of Isolated Guinea Pig Ileum Method are shown in Table No:4

C. Statistical analysis

Results obtained from each group were expressed as mean ± SEM. The data was analyzed by one way ANOVA followed by Dunnet's multiple comparison test9 for isolated guinea pig ileum preparation and Neuman Keul's test for histamine induced bronchoconstriction

III. RESULTS

Results of preliminary phytochemical screening of hydro alcoholic extract of prunus domestica fruits**:**

The Phytochemical Profile of The Extract[6]

Chemical constituents	Name of the test	Observations
Carbohydrates	Molich test	+ve
Glycosides	Borntrager's test	+ve
Fixed oils &fats	Saponification test	-ve
Proteins & free amino acids	Millons and Biuret test	+ve
Saponins	Foam test	-ve
Phenolic compounds	Dilute ferric chloride test	+ve

Phytosterol	Libermann – buchard's test	-ve
Alkaloids	Dragand roff's test	+ve
Flavanoids	Shinoda test	+ve

Table No: 1 Preliminary Phyto chemical Screening of Extract

Acute Toxicity Studies: OECD Guidelines

Acute Toxicity Studies:

Groups	Treatment	Behavioral changes & toxity	Mortality
1	100/mg/kg	None	Nill
2	300/mg/kg	None	Nill
3	500/mg/kg	None	Nill
4	1000/mg/kg	None	Nill
5	1500/mg/kg	None	Nill
6	2000/mg/kg	None	Nill

Table No: 2 Acute Toxicity Studies

Anti Histaminic Effect of All the 2 Test Extracts On PCT & LP

Groups	**Drug and Dose**	**PCT mean±SEM**	**LP mean±SEM**
Control	Distilled water	2.72±0.62	0.3±0.07
Standard	Chlorpheneramine 2mg/kg	12.16±0.34***	2.24±0.50*
Test-1	PD 200mg/kg	9.12±1.39***	1.29±1.56
Test-2	PD 400mg/kg	12.18±0.74	2.30±0.87

Values are expressed as mean±SEM (n = 6).***P < 0.1 when compared with Control**P < 0.5 when compared with Standard

Table No: 3 Values of Histamine Induced Broncho Constriction Method

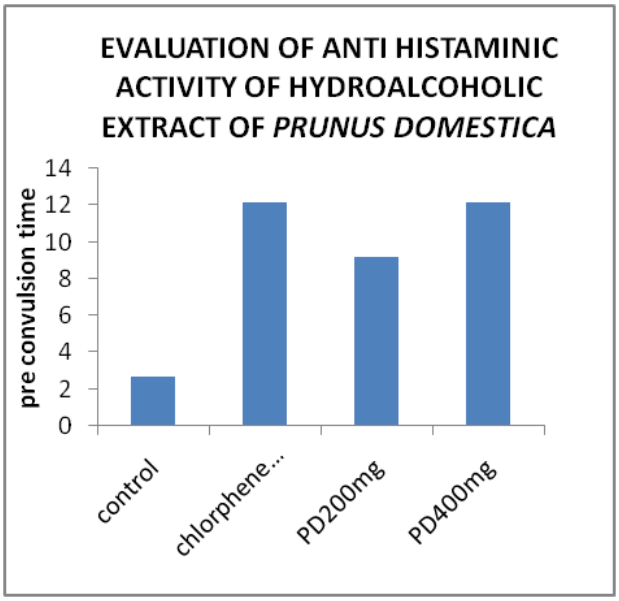

Fig.3.1 Graph for Pct

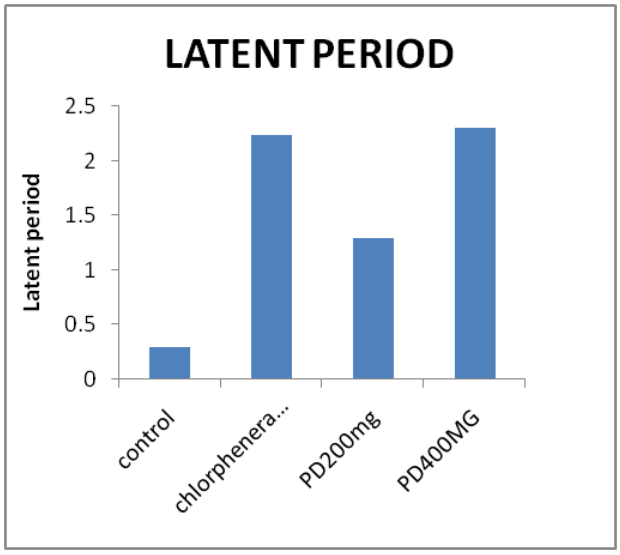

Fig.3.2 Graph for Latent Period

Anti Histaminic Effect of All the 2 Test Extracts On Guinea Pig Ileum

Group	Drug	Mean ± SEM
Control	Histamine	4.98±0.08
Standard	Chlophenearamine maleate	3.51± 0.04
Test-1	PD 200mg/kg	2.20± 0.04
Test	PD 400mg/kg	3.52 ±0.03

Values are expressed as mean±SEM (n = 6). **P < 0.1 when compared with Control*P < 0.5 when compared with Standard

Table No: 4 Values of Isolated Guinea Pig Ileum Method

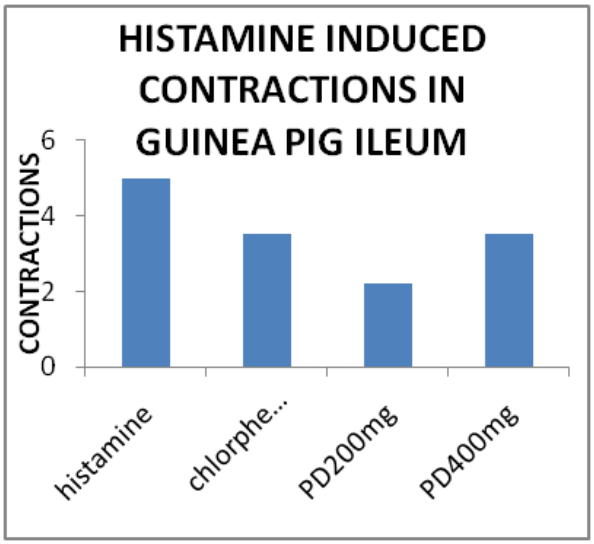

Fig.3.3 Contractions of Ileum of pig.

IV. DISCUSSION

In present study the effect of extract on histamine used contraction in Guineapig ileum produced as significant blocking at dose of 200mg\kg which is more than standard CPM and more than 400mg\kg extract with 'P' value <0.1Antihistaminic activity by histamine induced gunieapig has produced significant delay at 200mg\kg and 400mg\kg when compare to control group. 400mg\kg as produced an effect equal to CPM 2mg\kg with 'P' values <0.1 standard group has produced latent period of 2.424 min and test group 400mg\kg produce in 2.3 min .So it is definitely having H1 blocking activity and bronchodilator activity.

Prolongation of pre convulsion time and inhibition of contraction in gunieapig ileum by extract proves that it has antihistemic property mainly H1 receptor blocking. So it can be a potential drug to allergy,asthama, bronchoconstrictor like ailments .So it is further needed to carryout to identify the chemical constituent which is responsible for this activity.

V. ACKNOWLEDGEMENT

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ABBREVIATIONS USED

mg/ml – Milligram/Millilitre

µg/ml - microgram /milliliter

h - hour

min – minute

OECD: Organization for economic co-operation and development

LD – Loading dose

PCT – Preconvulsion time

Nacl- sodium chloride

Kcl – potassium chloride

Cacl2 – Calcium chloride.

Mgcl2 - Magnesium chloride

NaHco3 – Sodium bi carbonate

NaH2PO4 – Mono sodium phosphate

SEM – Standard error mean

LP - Latent period

REFFERENCES

[1] Sanchi Mehta, Neha Soni, Gouri Satpathy, Rajinder K. Gupta .Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of dried plum (Prunus domestica). Journal of Pharmacognosy and Phytochemistry. 2014; 3 (2): 166-171

[2] Naveen Dhingra1, Rajesh Sharma, Anand kar. Evaluation of the antioxidant activities of prunus domestica whole fruit: an in vitro study . International journal of pharmacy and pharmaceutical sciences .2014;6(4).

[3] Siamak Shahidi, Sara Setareye, and Minoo Mahmoodi.Effect of Prunus domestica L. (mirabelle) on learning and memory in mice,.Anc Sci Life. 2013;32(3): 139–143.doi: 10.4103/0257-7941.122996

[4] Soni, Manoj, Mohanty, p. k. jaliwala, y. a. Hepatoprotective activity of fruits of "prunus domestica.International Journal of Pharma & Bio Sciences.2011; 2(2):439-441

[5] Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur.Phytochemical screening and Extraction: A Review.Internationale pharmaceutica sciencia .2011 ;1(1)

[6] Khandelwal KR Practical Pharmacognosy, Techniques and experiments. 2nded, Nirali Prakashan Pune; 2004 .P.149-153

[7] Luque de Castro MD, García-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chimica Acta. 1998; 1(2):369-375.

[8] Dhirender koushi, Ruby rani, pawan koushik, dishasacher, Jyothi yadav.In vivo and in vitro Anti asthamatic study of piper longum linn, International journal of pharmacology, 2012,

[9] Rahul Hajare , V. M. Darvhekar , Ashish Shewale and Vijay Patil , Evaluation of antihistaminic activity of Piper betel leaf in guinea pig ,African Journal of Pharmacy and Pharmacology.2011; 5(2): 113-117.

[10] Kanakam Vijayabhaskar and Puram Paramesh. Evaluation of antihistaminic activity of benincasa hispida flower aqueous extract for histamine aerosol induces bronchoconstriction in guinea pig. world journal of pharmacy and pharmaceutical sciences.

[11] Bousquet J, Jeffery PK, Busse WW. Asthma: From bronchoconstriction to airways inflammation and remodeling. Am. J. Respir. Crit. Care Med. 2000;1(6):1749-1745.

[12] Uvnas B. Mast cells and histamine release. Ind J Pharmac.1969;1(1): 23-32.