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In vivo and *in vitro* Antiasthmatic Studies of Plant *Piper longum* Linn.

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Abstract: The fruits of *Piper longum* Linn. are used in allergic skin disorders and asthma. The effect of petroleum ether, alcoholic and decoction of the fruits of *P. longum* was studied for antihistaminic activity using Guinea pig ileum preparation (*in vitro*), histamine induced bronchospasm in Guinea pigs and haloperidol induced catalepsy in mice (*in vivo*). Its anti-allergic activity was evaluated using milk induced leukocytosis in mice and passive paw anaphylaxis in rats (*in vivo*). The extracts (100 μg mL^{-1}) significantly ($p<0.01$) inhibited the histamine induced contraction of isolated Guinea-pig ileum preparation. The extracts (50, 100, 200 mg kg^{-1}) showed the significant ($p<0.01$) activity and increase in dose of extract increased the % protection in histamine induced bronchospasm and also showed significant ($p<0.01$) activity in haloperidol induced catalepsy and passive paw anaphylaxis. In milk-induced leukocytes, petroleum ether and decoction extract (200 mg kg^{-1}) showed significant ($p<0.05$) decrease in number of leukocytes and alcoholic extract didn't show any significant effect.

Key words: Antiasthmatic, *Piper longum,* fruit

INTRODUCTION

Asthma literally means panting. Asthma is a common disease and its prevalence rising worldwide, with the highest prevalence in industrialized countries. 300 million people are affected by asthma worldwide and it has been estimated that it will further rise to 100 million by 2025 (Masoli *et al*., 2004; Bousquet *et al*., 2005). Asthma is recognized as a chronic lung disease by increased airway hyper-responsiveness and mucus production that leads to episodes of wheezing, coughing and shortness of breath (Annesi-Maesano, 2005). This may be due to liberation of endogenous and intrinsic mediators like bradykinin, chemokines, histamine, leukotrienes, nitric oxide, platelet activating factors and prostaglandins (Spina, 2000). *Piper longum* Linn. is a member of Piperaceae family. A slender climber with perennial woody roots is found both wild as well as cultivated, throughout the hotter parts of India from central to the north-eastern Himalayas. The herb also grows wild in Malaysia,. Singapore, Bhutan, Myanmar (Kirtikar and Basu, 1933). The various parts of the plant (fruits, roots, stems, leaves and seeds) are widely used by various tribal communities for the treatment of variety of ailments. The plant contains alkaloids, volatile oils, lignans and esters. For chronic bronchitis, cough and cold decoction of immature fruits was used. Roots and fruits are also used as anti-dote in snake biting and scorpion sting (Chopra *et al*., 1956).

A analysis of literature revealed some distinguished pharmacological activities of the plant such as analgesic (Vedhanayaki *et al*., 2003), anti-amoebic (Sawangjaroen *et al*., 2004; Ghoshal *et al*., 1996), anti-depressant (Lee *et al*., 2005), anti-fungal (Lee *et al*., 2001), anti-inflammatory, anti-microbial (Lokhande *et al*., 2007), hepatoprotective (Christina *et al*., 2006) and immunomodulatory (Sunila and Kuttan, 2004). There are no of plants which are found to be antiasthmatic (Malviya *et al*., 2011). Therefore, we investigated the anti-asthmatic activity of petroleum ether, alcoholic and decoction extract of the fruits of *Piper longum* using *in vitro* and *in vivo* models such as isolated Guinea pig ileum preparation, histamine-induced bronchospasm in Guinea pig, milk-induced leukocytosis in mice, haloperidol-induced catalepsy in mice and passive paw anaphylaxis in rats, respectively.

MATERIALS AND METHODS

Plant and preparation of extract: Fruits of *Piper longum* (5 kg) were purchased locally and authenticated by Dr. H.B. Singh, Scientist Incharge, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi where a voucher specimen (NISCAIR/RHMD/Consult/-2011-12/1751/51) has been deposited for further reference. The coarse powder of fruit (4 kg) were defatted with pet-

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ether (60-80°C) using a Soxhlet extraction method. The defatted plant material was then extracted with alcohol until it also become colourless. The solvents were evaporated under reduced pressure to obtain a semisolid mass and then vacuum dried to yield solid residues. Decoction of the fruit of *Piper longum* was also prepared by boiling of coarse powder (1 kg) of fruit with water for 12 h.

Animals: Dunkon-Hartley Guinea pigs (350-400 g), Wistar rats (150-250 g) and albino mice (20-25 g) of either sex were used for evaluating anti-asthmatic activity. Dunkon-Hartley Guinea pigs (350-400 g) of either sex bought from disease free animal house Chaudhary Charan Singh Haryana Agricultural University, Hisar. Wistar rats (150-250 g) and albino mice (20-25 g) of either sex bought from Animal House NIPER, Mohali. They were kept in the Animal House of Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra and housed at standard conditions of temperature (22±1°C) and 12/12 h light/dark cycle. They were fed with standard pellet diet (Ashirwad industries, Ropar, Punjab) and had free access to water. Fasting animals were used during the experiment. Permission for conduct of these experiments was obtained from IAEC.

Phytochemical screening of crude extract: Various chemical tests were carried out to identify the phytoconstituents as described by Khandelwal (2003).

Drugs and chemicals: Calcium chloride, dexamethasone, egg albumin, glucose, histamine, potassium chloride, sodium chloride, sodium dihydrogen phosphate, sodium hydrogen carbonate (Hi-Media, India), chlorpheniramine maleate and haloperidol (Ostar Lab, India), were used in this study.

Evaluation of anti-asthmatic activity using isolated Guinea pig ileum preparation: The Guinea pigs (overnight fasted) were sacrificed and the ileum was mounted in an organ bath containing Tyrode solution which was continuously aerated at 37±0.5°C. Bioassay of histamine 10 μg mL^{-1} in plain Tyrode solution and in Tyrode solution containing 100 μg mL^{-1} *Piper longum* Linn. extracts were performed. Percentage maximum contractile response was plotted to generate dose response curve of histamine, in the absence and presence of the plant extract (Pandit *et al*., 2008).

Histamine-induced bronchospasm: Dunkon-Hartley Guinea pigs were divided into 11 groups (n = 5). Control group received 5% tween 80 and other groups received single dose of pet-ether, alc. and decoction extracts (50, 100 and 200 mg kg^{-1} p.o.). CPM (2 mg kg^{-1}) was used as positive control. Prior to and after drug treatment each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. PCT was determined from the time of exposure to onset of dyspnoea leading to the appearance of preconvulsive dyspnoea in a sec. The % age protection offered by drugs in PCT was calculated for each dose and positive control was calculated by using the following formula (Kumar *et al*., 2010).

$$\text{Protection (\%)} = (1\text{-}T_1/T_2) \times 100$$

where, T_1 is the mean of PCT before administration of test drugs and T_2 is the mean of PCT after administration of test dugs.

Milk-induced leukocytosis: Albino mice were divided into 11 groups (n = 5). Control group received 5% tween 80 and other groups received single dose of pet.ether, alc. and decoction extracts (50, 100 and 200 mg kg^{-1} p.o.). Only Milk received group served as an intoxicant. After 1 h of drug treatment except control all groups received boiled and cooled milk injection in dose of 4 mL kg^{-1} (s.c.). Total leukocyte count was done in each group before drug administration and 24 h after milk injection.

Haloperidol-induced catalepsy: Albino mice were divided into 11 groups (n = 5). Control group received 5% tween 80 and other groups received single dose of pet.ether, alc. and decoction extracts (50, 100 and 200 mg kg^{-1} p.o.). CPM (10 mg kg^{-1}) was used as positive control. The entire group received haloperidol (1 mg kg^{-1} i.p.). 1 h after the drug administration and the duration of catalepsy was measured at 0, 30, 60, 90, 120 and 150 min (Pandit *et al*., 2008).

Passive paw anaphylaxis: Wistar rat were given subcutaneously three doses of 100 μg of egg albumin (s.c.) on day 1st, 3rd and 5th. On 10th day of sensitization, blood was collected from the retro orbital plexus and collected blood was allowed to clot and the serum was separated by centrifugation at 1500 rpm. Animals were divided into 11 groups (n = 5). Control group received 5% tween 80 and other groups received single dose of pet-ether, alcoholic and decoction extract (50, 100, 200 mg kg^{-1} p.o.). Dexamethasone was used as standard (0.27 mg kg^{-1} p.o.). Prior to drug treatment animals were sensitized with serum. Next 24 h, after drug treatment animals again challenged with 10 μg egg albumin. The difference in the reading prior to and after antigen challenge represented the edema volume and percent inhibition of volume was calculated by using the following formula (Kulkarni *et al*., 2010).

Percent inhibition = 1- (Vt/ Vc) ×100
Vt = Mean relative change in paw volume in test group
Vc = Mean change in paw volume in control group

Statistical analysis: All values were expressed as mean±SEM and data were analyzed by ANOVA followed by Dunnett's t-test.

RESULTS

Phytochemical screening: The petroleum ether extract (PF), alcoholic extract (AF) and decoction (DF) of plant *Piper longum* Linn. contains alkaloids, carbohydrates, flavonoids, glycosides and steroids except alkaloids in alcoholic and decoction.

Evaluation of anti-asthmatic activity using isolated Guinea pig ileum preparation: In the present study pet.ether, alc. and decoction extracts of fruits of *Piper longum* (100 μg mL^{-1}) significantly ($p<0.01$) inhibited the histamine induced contraction of isolated Guinea-pig ileum preparation indicating its H_1 receptor antagonistic activity and supports the anti-asthmatic properties of the plant. There was decrease in % response in the presence PF, AF and DF at dose of 100 μg mL^{-1} of *Piper longum* when compared to histamine (10 μg mL^{-1}) alone. The observations are given in Table 1.

Histamine-induced bronchospasm: The extracts of *Piper longum* at dose of 50, 100 and 200 mg kg^{-1} p.o. exhibited significant prolonged the latent period of convulsion followed by exposure to histamine aerosol (0.2%). The maximum percentage protection i.e., 83.33% observed at 200 mg kg^{-1} dose of PF for bronchorelaxant study comparable with that of standard CPM (2 mg kg^{-1}) 88.36% (Table 2).

Milk-induced leukocytosis: In the milk-induced leukocytes count, dose of 200 mg kg^{-1} body weight of PF and DF showed significant ($p<0.05$) activity decrease in number of leukocytes (1280.0±0.81 and 1400.0±0.97) as compared to control group (4100±0.22). The AF didn't show any significant effect (Table 3).

Haloperidol-induced catalepsy: In measurement of catalepsy by block method all extracts at all doses showed significant ($p<0.01$) activity at all time interval. The AF at dose 100 mg kg^{-1} was most effective at 30 and 60 min time interval with values 93±1.21 and 61±1.34, PF 200 mg kg^{-1} at 90 min with value 79±1.81, AF 200 mg kg^{-1} at 120 min with value 80±1.66 and DF 200 mg kg^{-1} at 150 min with value 98±1.08 showed most significant ($p<0.01$) activity as shown in Table 4.

Passive paw anaphylaxis: In passive paw anaphylaxis model, PF at dose of 50, 100 and 200 mg kg^{-1} body weight showed significant ($p<0.01$) activity at 1 h. After 2 h all extracts showed significant ($p<0.01$) activity. After 3 h PF at dose of 100 mg kg^{-1} and 200 mg kg^{-1} body weight showed significant ($p<0.01$) activity and AF at dose of 200 mg kg^{-1} body weight showed significant ($p<0.05$) activity. The DF didn't show any significant effect (Table 5).

Table 1: Effect of various extracts of *Piper longum* Linn. on isolated Guinea pig ileum preparation

		Response (%)					
		0.1	0.2	0.4	0.8	1.6	3.2
Groups	Drug dose	(mL)					
Control	Histamine (10 μg mL^{-1})	22.50±0.058	40.0±0.088	85.00±0.116	90.00±0.089	97.5±0.058	100.00±0.08
Standard	Histamine+CPM (10+10 μg mL^{-1})	1.00±0.003**	0.90±0.007**	0.83±0.009**	0.83±0.009**	4.7±0.011**	7.25±0.007**
PF	Histamine+PF (10+100 μg mL^{-1})	1.00±0.007**	1.00±0.006**	7.30±0.019**	9.75±0.009**	12.5±0.058**	22.50±0.058**
AF	Histamine+AF (10+100 μg mL^{-1})	9.25±0.027**	2.25±0.007**	5.00±0.012**	11.25±0.029**	30.0±0.029**	40±00.044**
DF	Histamine+DF (10+100 μg mL^{-1})	4.75±0.009**	16.30±0.005**	22.50±0.058**	30.75±0.089**	33.0±0.044**	40.70±0.033**

Values are in Mean±SEM, One-way ANOVA followed by Dunnett's t-test, Where* $p<0.05$ and **$p<0.01$ as compared to control, n = 5

Table 2: Effect of various extracts of *Piper longum* Linn. on histamine induced bronchospasm

Groups	Drug dose, route	PCT (before)	PCT (after)	Mean exposition time	Protection (%)
Control	Tween 80 (10 mL kg^{-1} p.o.)	85±1.09	88±1.14	3±0.63	3.41
Standard	CPM (2 mL kg^{-1} p.o.)	56±1.55	481±0.84	425±1.19**	88.36
PF	50 mL kg^{-1} p.o.	55±1.52	124±1.09	69±1.41**	55.65
AF	50 mg kg^{-1} p.o.	74±1.38	120±0.84	46±1.82**	38.33
DF	50 mg kg^{-1} p.o.	55±1.30	115±0.89	60±1.14**	52.17
PF	100 mg kg^{-1} p.o.	74±1.67	224±1.14	150±2.35**	66.96
AF	100 mg kg^{-1} p.o.	64±0.63	240±1.05	176±1.67**	73.33
DF	100 mg kg^{-1} p.o.	64±0.89	393±0.89	160±1.23**	71.43
PF	200 mg kg^{-1} p.o.	64±1.05	384±1.30	320±2.09**	83.33
AF	200 mg kg^{-1} p.o.	73±0.89	393±0.89	320±1.25**	71.43
DF	200 mg kg^{-1} p.o.	62±1.05	325±1.14	261±1.95**	80.80

Values are in Mean±SEM, One-way ANOVA followed by Dunnett's t-test, Where* $p<0.05$ and **$p<0.01$ as compared to control, n = 5

Table 3: Effect of various extracts of *Piper longum* Linn. on milk induced leukocytosis

Groups	Drug dose, route	No. of leukocytes (Cu.mm)
Control	5%Tween 80 (10 mL kg^{-1})+Milk (4 mL kg^{-1} s.c.)	4100.0±0.22
Standard	CPM (2 mg kg^{-1}), i.p. + Milk (4 mL kg^{-1} s.c.)	600.0±0.02**
PF	50 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	3480.0±0.04
AF	50 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	3140.0±0.12
DF	50 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	2200.0±0.75
PF	100 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	2580.0±0.68
AF	100 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	2920.0±0.33
DF	100 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	1900.0±0.22
PF	200 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	1280.0±0.81*
AF	200 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	1960.0±0.47
DF	200 mg kg^{-1} p.o.+Milk (4 mL kg^{-1} s.c.)	1400.0±0.97*

Values are in Mean±SEM, One-way ANOVA followed by Dunnett's t-test, Where* $p<0.05$ and **$p<0.01$ as compared to control, n = 5

Table 4: Effect of various extracts of *Piper longum* Linn. on haloperidol induced catalepsy

		min				
Groups	Drug (Dose), route	30	60	90	120	150
Control	5% Tween 80 (10 mL kg^{-1})+Haloperidol (1 mg kg^{-1} i.p.)	586±1.92	887±1.14	777±1.91	781±1.25	589±1.99
Standard	CPM (2 mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1} i.p.)	80±0.93**	62±1.36**	61±1.43**	72±1.29**	62±1.52**
PF	50 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1} i.p.)	265±1.24**	360±1.41**	185±1.97**	221±1.57**	197±1.49**
AF	50 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1} i.p.)	141±1.39**	112±1.94**	142±1.54**	359±1.00**	190±2.04**
DF	50 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1} i.p.)	556±2.67**	187±2.21**	207±2.29**	137±1.60**	367±1.72**
PF	100 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1} i.p.)	156±1.50**	170±1.60**	134±1.89**	123±1.61**	174±1.60**
AF	100 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1} i.p.)	93±1.21**	61±1.34**	133±1.36**	123±1.69**	372±1.08**
DF	100 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1} i.p.)	340±1.16**	169±1.00**	164±2.17**	173±1.33**	249±1.21**
PF	200 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1}), i.p.	105±1.99**	94±1.12**	79±1.81**	110±1.63**	118±2.07**
AF	200 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1}), i.p.	86±1.39**	61±1.54**	107±1.72**	80±1.66**	145±2.15**
DF	200 (mg kg^{-1} i.p.)+Haloperidol (1 mg kg^{-1}), i.p.	323±2.15**	160±1.11**	76±1.75**	159±2.16**	98±1.08**

Values are in Mean±SEM. One-way ANOVA followed by Dnnnett's t-test, Where* $p<0.05$ and **$p<0.01$ as compared to control

Table 5: Effect of various extracts of *Piper longum* Linn. on passive paw anaphylaxis (Mean decrease in paw volume)

		h			
Groups	Drug (Dose), route	1	2	3	4
Control	Tween 80 (10 mL kg^{-1} p.o.)+EA (10 µg s.c.)	0.63±0.03	0.56±0.02	0.53±0.03	0.43±0.01
Standard	Dexamethasone (0.27 mg kg^{-1} i.p.)+EA (10 µg s.c.)	0.25±0.02**	0.11±0.01**	0.25±0.01**	0.22±0.05*
PF	50 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.36+0.06**	0.21+0.01**	0.39+0.04	0.32+0.08
AF	50 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.46+0.059	0.26+0.08**	0.49+0.03	0.31+0.06
DF	50 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.43+0.06	0.27+0.01**	0.39+0.0	0.32+0.07
PF	100 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.30+0.05**	0.21+0.01**	0.26+0.01**	0.26+0.02
AF	100 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.42+0.06	0.25+0.02**	0.38+0.01	0.29+0.05
DF	100 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.38+0.04*	0.21+0.03**	0.37+0.08	0.28+0.03
PF	200 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.29+0.08**	0.20+0.06**	0.25+0.02**	0.25+0.02
AF	200 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.30+0.04**	0.22+0.04**	0.32+0.05*	0.27+0.02
DF	200 mg kg^{-1} p.o.+EA (10 µg s.c.)	0.37+0.02*	0.21+0.03**	0.36+0.01	0.27+0.01

Values are in Mean±SEM, One-way ANOVA followed by Dunnett's t-test, Where* $p<0.05$ and **$p<0.01$ as compared to control, n = 5

DISCUSSION

The present study deals with screening of anti-asthmatic activity of PF, AF and DF of fruit of *Piper longum* Linn. using *in vitro* and *iv vivo* models such as isolated Guinea pig ileum preparation (*in vitro*), histamine-induced bronchospasm in Guinea pigs, milk-induced leukocytosis in mice, haloperidol-induced catalepsy in mice and passive paw anaphylaxis in rats (*in vivo*).

Bronchial asthma is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyper responsiveness to various stimuli, in which many cell types play a role, more important being mast cells, eosinophils and T-lymphocytes . Different agonist like, ACh, histamine, 5-HT and bradykinin are responsible for contractile responses (Tripathi, 2003; Ghosh, 2005). Guinea pig ileum is used for screening of anti-histaminic activity. The stimulation of H_1 produces graded dose related contraction of isolated Guinea pig ileum. In the present study pet.ether, alc. and decoction extracts of fruits of *Piper longum* (100 µg mL^{-1}) significantly ($p<0.01$) inhibited the histamine induced contraction of isolated Guinea-pig ileum preparation indicating its H_1 receptor antagonistic activity and supports the anti-asthmatic properties of the plant. There was decrease in % response in the presence PF, AF and DF at dose of 100 µg mL^{-1} of *Piper longum* when compared to histamine (10 µg mL^{-1}) alone.

Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing airway hyper responsiveness and bronchial airway inflammation

(Ghosh, 2005). Histamine induced bronchoconstriction is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled 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 (Nayampalli *et al*., 1986). In histamine induced bronchospasm all extracts showed the significant ($p<0.01$) activity and increase in dose of extract increased the % protection. The maximum percentage protection i.e., 83.33% observed at 200 mg kg^{-1} dose of PF for bronchorelaxant study comparable with that of standard CPM 88.36%. The results of the study confirmed the bronchodilator properties of the plant, justifying its traditional claim in the treatment of asthma.

Herbal formulations used in the treatment of asthma include some anti-stress herbs to enable adoption to stress since excessive stress or nervous debility may aggravate symptoms of asthma. The normalization effect of an adaptogen can be observed in milk-induced leukocytosis (increase in leukocyte count) after parental administration of milk. In the milk-induced leukocytes count, dose of 200 mg kg^{-1} body weight of PF and DF showed significant ($p<0.05$) decrease in number of leukocytes. The AF didn't show any significant effect.

Haloperidol induces catalepsy by inhibiting dopamine D_2 receptors and inhibits dopamine secretion. Dopamine is agonist for adrenaline. Adrenaline is physiological antagonist of histamine. So as there decrease in dopamine there is imbalance in neurotransmitters means high level of histamine (Tripathi, 2003). In haloperidol induced catalepsy all extract showed significant ($p<0.01$) activity.

Allergic asthma is a chronic inflammatory process occurring due to exposure of allergen resulting in the activation of T-lymphocytes with subsequent release of inflammatory mediators. Immuno-modulating agents are useful in the treatment of asthma by inhibiting the antigen-antibody (AG-AB) reaction and there by inhibiting the release of inflammatory mediators (Tripathi, 2003). *Piper longum* has been reported to possess anti-inflammatory activity. In passive paw anaphylaxis, all the extracts of *Piper longum* showed significant ($p<0.01$) activity at 2 h. Percent inhibition of paw oedema volume was calculated and maximum effective dose was observed at 200 mg kg^{-1} of PF at different hour intervals. Whereas, in statistical analysis of paw oedema volume it was observed that 200 mg kg^{-1} dose of PF had significant ($p<0.01$) effect comparable that with dexamethasone. Here also observed that increase in dose of all extracts increased activity.

Drugs effective in the asthma are mostly steroidal in nature. Phytochemical analysis of the extracts of the fruit of *Piper longum* Linn. showed the presence of the alkaloids, steroids, glycosides, flavonoids and carbohydrates. The pet.ether, alc. and decoction extracts are effective in all the models of asthma as order of PF> AF>DF except AF in milk-induced leukocytosis. Therefore it can be concluded that the plant *Piper longum* is said to possess the anti-asthmatic activity.

CONCLUSION

Drugs effective in the asthma are mostly steroidal in nature. Phytochemical analysis of the extracts of the fruit of *Piper longum* Linn. showed the presence of the alkaloids, steroids, glycosides, flavonoids and carbohydrates. The PF, AF and DF are effective in all the models of asthma as order of PF>AF>DF except alcoholic extract in milk-induced leukocytosis. Therefore it can be concluded that the plant *Piper longum* is said to possess the anti-asthmatic activity.

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