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RESEARCH ARTICLE

Formulation and Biological Evaluation of Some Selected Medicinal Plants for Anti-inflammatory Potential

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ABSTRACT

Objective: The aim of the paper is to assess the anti-inflammatory potential of three medicinal plants using two rat models. **Materials and Methods:** Soxhlet extraction approaches utilized to separate the constituents of interest. Quantitative analysis has been performed to determine the total phenolic and flavonoid content. Three plants extract employed for the ointment formulation by addition of the extract of *Artocarpus heterophyllus* (AH), *Murraya koenigii* (MK), and *Punica granatum* (PG) in polyethylene glycol (PEG) ointment base, a blend of PEG 600 and PEG 4000, and ratio 7:3, respectively. Two rat models based on chemical induced animals employed for the anti-inflammatory potential. **Results and Discussion:** All three plants including AH Lam., MK Linn., and PG Linn. extracted for the major component and have shown the gallic acid and quercetin as major component for flavonoid and phenol content. The ointment formulation F3 has showed maximum inhibition (80.95%) at 50 mg/kg dose of carrageenan-induced edema and 83.33% inhibition at 100 mg/kg dose. The ointment formulation F3 has showed maximum inhibition (78.57%) at 50 mg/kg dose of histamine persuade edema and 83.33% inhibition at 100 mg/kg dose. F3 ointment formulation is better than the F2 and F1 formulation in inhibition and in all phases showing its reserve of kinins as well as arachidonic acid. **Conclusion:** Quantitative and pharmacological evaluation indicated that ointment formulations of AH, MK, and PG have exploit for anti-inflammatory activity. The normal extract has shown the least activity but ointment formulations have shown the better result. The ointment formulations containing plant extracts in 10% amount have better wound healing potential.

Keywords: Anti-inflammatory, carrageenan, gallic acid, histamine, quercetin

INTRODUCTION

When human body affects by the any disease, inflammatory process guard the body through stimulation of chemicals and mediators that prevent the infection by combat foreign substances.[1] These processes are necessary for optimal repair and immune surveillance and revival of following injury.[2] Inflammation is denoted seeing that chronological liberation of the mediators including bioactive amines, eicosanoids, cytokines, chemokine, and growth factors that regulate and increased vascular permeability.[3] Pain is external and expressive incident that directly related to the inflammation.[4] Enhanced production of prostaglandins is directly connected by way of the pain, fever, and inflammation[5] and these gush cause several disease defects includes arthritis, inflammatory bowel disease, and psoriasis.

Rheumatoid arthritis and degenerative arthritis are the major inflammatory diseases that affects people worldwide and the mediators that generated in inflammation process reaches to the circulation and may the reason behind the fever and pain.[6] These mediators also stimulate the immune cells that activate the signal to enhance the migration capacity to inflamed tissue and made clusters that formed by immune systems and combination of

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endothelial and inflamed cells. Many steroids, specifically glucocorticoids, and mineralocorticoid reduce swelling by binding to corticoid receptors. Long-term uses have several severe side effects, for example, hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis, and anxiety effects.[7]

Natural ingredients that extracted from plant resource are the rich sources of terpenes, catechin, alkaloid, glycosides, phenols, and anthoxanthin and shown the potential candidates against inflammation and arthritis. Plant resources contains the phyto-constituentsincludesalkaloid(Thalicsiline,cycleanine and tetranidrine, trilobine and isotrilobine),[8,9] Terpenoids (Aescin (β-amyrin, chiisanosides, lupine, dysobinin, boswellic acid),[10] Favonoids (Quercetin-3-orhamnoglucoside, kaempferol, hedychinone, marmin coumarin),[11] Saponin and sapogenins (Phytolaccoside B, misaponins, saikogenin, glycyrrhytinic and glycyrrhizinic acids) and all these have shown the anti-inflammatory as well as wound healing potential.[12]

From moment in ancient time, plants are used as medicine in universe and natural resources based remedy have been the mainstay of traditional societies in dealing with health problems. The WHO stated that about 80% of earth population based on usual remedy for their primary strength and rehabilitation is focused on natural ingredients and their active principles. Various plant drugs may produce their anti-inflammatory actions by various proposed mechanisms as follows, reticence of cyclooxygenase enzyme (COX I and II), reticence of leukocyte migration to the site of inflammation, arachidonic acid synthesis, and block the release of histamine from mast cells.

The objective of the research paper is to assessment of anti-inflammatory potential of three medicinal plants includes *Artocarpus heterophyllus* (AH) Linn., *Murraya koenigii* (MK) Linn., and *Punica granatum* (PG) Linn. All these plant has also shown the wound heal potential.[13]

MATERIALS AND METHODS

Plant material collection and authentication

The pulp of AH, whole plant of MK and bark of PG was collected and procured from Vidisha district, Madhya Pradesh. The plants sample was composed and recognized as pulp of AH, bark of PG, MK, and authenticated by Dr. Zia ul Hassan, Department of Botany, Saifia Science College, Bhopal. The appension no. for the specimen is 498/BS/saifia/NI_MK_PG_AH/04/16/07 and has been preserved for future identification. The specimen was dried at room temperature.

AH is recognized as jackfruit belong to Moraceae family.[14] The AH [Figure 1] contains various chemical constituents, i.e., artocarpin, artocarpetin, norartocarpetin, atropine, cycloartinone morin, dihydromorin, cynomycin, and artocarpanone.[15]

The AH may contains albumin 1.7%, cellulose 59.0%, lipid 1.7%, moisture 6.7%, and glycosides 38.0%,[16,17] root contains β-sitosterol, ursolic acid, betulinic acid, and cycloartenone,[18] and latex is used as an anti-inflammatory agent.[19]

MK is identified as curry leaf [Figure 2]. The oil obtained from the leaves used as antioxidant, anthelmintic,[20] and antibacterial properties,[21] increase digestive secretions; relieve nausea, indigestion, and vomiting and used internally in treating constipation, colic, antimicrobial,[22] and diarrhea.[23]

PG is known in Hindi as Anardana [Figure 3], basis of the many nutrients and many agrochemical applications. This fruit contains the sub-acid flavor juice and a high sources of the protein, fat, and carbohydrate.[24,25] The flowers used for the treatment of dysentery, stomach ache, and cough. PG is also used as antidiabetic,[26] antioxidant, anti-inflammatory, and antiproliferation.[27,28]

Figure 1: Fruit of *Artocarpus heterophyllus* (Kathal)

Extraction

The pulp of AH, bark of PG, and leaves of MK were separated from the fresh and dried at room temperature. The shade-dried, coarsely powdered materials (300 g) were defatted by petroleum ether (43°C). The extract was obtained using Soxhlet extraction. The extracts were dried under reduced pressure at low temperature to have different mixture of constituents.

Quantitative analysis

Determination of total phenol content

Folin–Ciocalteu method has been utilized for the determination of phenol content. Standard curve of the gallic acid was prepared using different dilution (0.8, 1.6, 3.12, 6.23, 12.3, and 23 μg/ml). The absorbance was recorded after 90 min at 760 nm UV/visible spectrophotometer. The phenol content was calculated as gallic acid equivalents (mgGAE/g). All process were repeated in duplicate and regression equation used to calculate total phenole content of the extracts.[17]

Figure 2: Leaf of *Murraya koenigii* (curry leaf)

Figure 3: Fruit of *Punica granatum* (Anardana)

Determination of total flavonoid content

The standard curve of quercetin was prepared by taken 10, 20, 30, 40, and 50 μg/ml concentrations and 1 M sodium hydroxide was added sequentially. The absorbance was recorded at 310 nm on UV-spectrophotometer. The similar process was repeated and total flavonoid content was calculated as quercetin equivalents (mgQE/g). All experimental were performed in duplicate and expressed as average of two analysis.

Preparation of ointment formulations

The extracts of three plants have been taken and ointment formulation was formulated and prepared by their ease of preparation and cleaning after application.

Ointment formulation

Polyethylene glycol (PEG) mixture of PEG 4000 and 600 has utilized for ointment formulation, with concentration of 10% (w/w) of all plants extracts. Ointment was formulated by fusion method.[29] The prepared ointment was then evaluated by various parameters, for example, consistency, and stability.[27,30,31]

The plants extract utilized for the ointment preparation is depicted in Table 1.

Evaluation of formulation

1. Color and Odor: Physical parameters such as color and odor were examined by visual examination.
2. pH: pH of ointment formulation was measured by digital pH meter. The solution of ointment was prepared using 100 ml of distilled water and set aside for 2 h. pH was determined in triplicate and average value was calculated.
3. Spreadability: The spreadability was determined using two slides to get uniform

Table 1: Ointment formulation based on three plant extract

S. No.	Content	Formulation code
1	*Murraya koenigii* whole plant extract (MK) (10%) + Ointment base	F1
2	*Punica granatum* bark extract (10%) (PG) + Ointment base	F2
3	*Artocarpus heterophyllus* pulp extract (10%) (AH) + Ointment base	F3

thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. The better spreadability counts when slightly less time taken for separation of the sides.[32] Spreadability was calculated by following formula: S = M×L/T; Where, S = Spreadability, M = Weight tide to the upper slide, L = Length of glass slide, and T = Time taken to separate the slides.

4. Extrudability: The extrudability was evaluated, as reported by Sawant and Tajane, 2016.[29]
5. Diffusion study: Agar nutrient medium was make the most of the diffusion study. The time taken by ointment to get diffused through was noted.
6. Loss on drying (LOD): LOD was determined using Petri dish and dried for the temperature 103°C.
7. Solubility: The solubility of formulation checked in boiling water, alcohol, ether, and chloroform.
8. Washability: Ointment formulation was applied on skin and washed with water an ease was checked.
9. Non irritancy test: Ointment applied to the skin and observed for the effect.
10. Stability study: Stability test of the ointment was carried out for 28 days at various temperature conditions.[33,34]

Pharmacological screening

Anti-inflammatory activity

The various extracts and ointment formulation F1, F2, and F3 were taken for the pharmacological screening to identify fractions responsible for anti-inflammatory activity. Screening carried out by two animal models, i.e., (1) carrageenan persuade paw edema in rats; and (2) histamine persuade paw edema in rats.[35]

Determination of LD_{50} value and acute toxicity

Rats in groups of 12 were administered intraperitonealy with different doses of the fractions from the three drugs by the staircase method, starting from 10 mg/kg, and increasing dose with factor 2.0, if there was no mortality and decreasing subsequent dose with factor 0.7[36] in case there was mortality. Least and most tolerated were determined by hit and trial method for various extracts and fractions. Corrections for 0% and 100% mortality were done by the formulas: 100 (0.25/n); for 0% mortality: and, 100 (n − 0.025/ n); for 100% mortality. S.E. of LD_{50} = Log dose with highest mortality−Log dose with lowest mortality/n.

Doses were selected among two and any mortality experimental for 24 h and the number of deaths noted. A curve of log dose versus probit assessment was plotted to get dose for probit value 5 which was taken to be LD_{50}. Dose range well below LD_{50} was selected for study.

Screening for anti-inflammatory

Inhibition of carrageenan induced, paw edema in rats

Rats were divided in the groups of six each, total 12 groups are created (120–150 g):

(1) Saline treated control, (2) untreated diseased animals, (3) reference group (indomethacin) before carrageenan, and (4) experimental groups.

Control Group I was given saline, 1 h earlier than the carrageenan infusion. Experimental groups were given doses of different portions in 0.5 ml of ordinary saline, infused intraperitonealy 1 h before infusion of 0.1 ml of 1% carrageenan arrangement in the right rear paw under the plantar aponeurosis (s.c) for affectation of edema. The quantity of paw edema was controlled by Plethysmometer and a measurement reaction relationship was built for both oral and i.p. dosage[37] and a connection built between i.p., furthermore, oral dosages delivering most extreme mitigating impact. Reference gathering was given indomethacin 2.5 mg/kg 1 h preceding the carrageenan infusion. Rate restraint of edema in respect to a control gathering was ascertained.

Inhibition of histamine paw edema in rats

In this experiment, histamine (0.1 ml of 1 mg/ml) was used as phlogistic agents.[38] The extract and ointment with various fractions, standard pyrilamine, and control vehicle (2.5%, DMSO, and Tween 20) were regulated intraperitonealy 1 h earlier than the infusion of incendiary arbiters in their particular gatherings. Various doses of concentrate or fractions were infused intraperitonealy in vehicle to find out dosage reaction relationship.[39] Histamine (1 mg/ml) was infused and reaction noted 60 min for histamine bunches. Pyrilamine maleate (1 mg/kg) was utilized as the reference of histamine and as a standard medication in the reference bunch. The quantity of paw edema was dictated by plethysmometer.

RESULTS AND DISCUSSION

Extraction

The extraction was done by continuous hot percolation method, i.e., Soxhlet apparatus. The dried and pulverized drug was utilized with petroleum ether. Procured marc was then extracted with 70% solution. The extract was dried by rota vacuum evaporator. The percentage yield of the extract from different plants is tabulated in Table 2.

Quantitative analysis

Determination of total phenol content

Spectrophotometric method with Folin-Ciocalteu reagent utilized for the determination of total phenol in various plant extracts. The concentration of total phenols in different plant extracts is in the following ascending order: MK < AH < PG.

The extract of PG Linn. has shown that high concentration of total phenol content (64.167 mg GAE/g) as compared to the extract of AH Linn., (53.889 mg GAE/g) and hydroalcoholic extract of MK Linn., MK (22.50 mg GAE/g) has shown the least phenolic content. The total phenol was significantly more in the hydroalcoholic extract of PGB and AHP as compared to MKL. The extract from pulp of AH Linn has also more phenol about more than 2.4 times than the whole plant extract of MK Linn. It is evident from the data that the bark and fruit whole plant has more phenol content than the leaves in the extracts.

Table 2: Percentage yield of the extracts from plants

S. No.	Part	Extract color	Yield (in g)
1	AH	Yellowish	8.24
2	PG	Dark brown	8.97
3	MK	Greenish brown	20.76

Initially 80 g of crude drug was taken. Where AH – Hydroalcoholic extract of *Artocarpus heterophyllus* pulp, PG – Hydroalcoholic extract of PG Linn. Bark, and MK – Hydroalcoholic extract of *Murraya koenigii* Linn. whole plant

Determination of total flavonoid content

The concentration of flavonoids in various extracts of plants parts was determined UV spectrophotometric method with aluminum chloride. The concentration of flavonoids in different plant extracts is in the following ascending order: MK < AH < PG. The hydroalcoholic extract of the bark of PG Linn. has shown high concentration of total flavonoid content (775.76 mgQE/g) as compared to others. Concentration dependent flavanoidal content variation has validated the analytical methodology, for example, UV/visible spectroscopy. The total flavanoidal content was more in the hydroalcoholic extract of PG (775.76 mgQE/g) when compared AH (617.87 mgQE/g) and MG (722.27 mgQE/g).

Preparation and characterization of ointment formulations

Ointment formulation F1, F2, and F3 contains of MK Linn extract, PG Linn. Extract, and AH Lam, respectively. The herbal extracts of MK Linn extract, PG Linn., and AH Lam were obtained from continuous hot percolation extraction method in good yield. PEG ointment base, blend of PEG 600 and 4000 utilized for ointment formulation by taken 10% (w/w) concentration of extracts. The ointment was estimated for various physicochemical parameters and is presented in Table 3. This ointment estimated for a period of 3 months and result indicated that no sign of any deviation was observed in physical appearance, pH, and rheological properties. These formulations also tested for the irritant

effect and concluded that these formulations have free from irritation. The ointment formulations depicted the absence of redness, inflammation, and edema.

Anti-inflammatory activity

Various extracts were subjected to detailed pharmacological investigations for anti-inflammatory activity along with determination of toxicity.

Inhibition of carrageenan-induced paw edema in rats

It is reported that carrageenan induce the inflammation by escalating PE2 release and leukocytes migration. It is moreover increase the COX-2 appearance in skeletal muscle and epidermis suggests the production of prostaglandin E2. Inflammation induced through carrageenan engross three continuous phases of the discharge of the mediator; as well as serotonin and histamine in the primary phase (0–2 h); kinin released in secondary (3 h); and PG in the 3rd phase (>4 h). The separated extract and ointment formulation from the three plants were evaluated for capacity to inhibit carrageenan induced edema at different doses given intraperitonealy. The observed inhibitions are tabulated in Table 4. The ointment formulation F3 has shown maximum inhibition (80.95%) at 50 mg/kg dose of carrageenan-induced edema [Table 4] and 83.33% inhibition at 100 mg/kg dose [Table 4], followed by MK5 and MK10 from MK (38.09 and 47.61% approximately) ($P < 0.001$ for all) [Tables 4]. The PG5 and PG10 from PG Linn. extract were shown 50% and 57.14% of inhibition at 100 mg/kg dose but in case of 50 mg/kg dose it inhibits only 47.61% and 54.76%, respectively. The F1 and F2 ointment formulation has shown 76.19% and 80.95% of inhibition at 100 mg/kg dose but in case of 50mg/kg dose it inhibits only 73.80% and 76.1%, respectively. The AH5 and AH10 from AH Lam extract were shown 64.28% and 71.42% of inhibition at 100 mg/kg dose but in case of 50 mg/kg dose it inhibits only 59.62% and 64.28%, respectively.

Inhibition of histamine-induced paw edema in rats

The separated fractions from the three plants were evaluated to inhibit histamine-induced edema

Table 3: Ointment formulation with their physicochemical characterization

S. No.	Parameters	Formulations		
		F1	F2	F3
1	Color	Yellowish brown	Dark yellowish brown	Yellowish brown
2	Odor	Characteristic	Characteristic	Characteristic
3	Consistency	Smooth	Smooth	Smooth
4	pH	5.9±0.1	6.4±0.1	6.1±0.1
5	Spreadability (s)	7.2±0.38	8.6±0.25	6.5±0.24
6	Extrudability (g)	0.41±0.06	0.47±0.11	0.39±0.04
7	Diffusion study (after 60 min)	0.70±0.04	0.65±0.05	0.65±0.02
8	Loss on drying (%)	30.24±1.20	30.73±1.31	29.14±1.11
9	Solubility	Soluble in boiling water, miscible with ethanol, ether, chloroform	Soluble in boiling water miscible with ethanol, ether, chloroform	Soluble in boiling water miscible with ethanol, ether, chloroform
10	Washability	Good	Good	Good
11	Non-irritancy	Non-irritant (Absence of redness, rashes, edema)	Non-irritant (Absence of redness, rashes, edema)	Non-irritant (Absence of redness, rashes, edema)
12	Stability studies	Stable	Stable	Stable

Whereas F1 (MK extract (10%) + Ointment base); F2 (PG extract (10%) + Ointment base); F3 (AH extract (10%) + Ointment base). MK Linn. extract, PG Linn. and AH Lam.

at different doses given intraperitonealy. The observed inhibitions are tabulated in Table 5.

The ointment formulation F3 has shown maximum inhibition (78.57%) at 50 mg/kg dose of histamine-induced edema and 83.33% inhibition at 100 mg/kg dose. The MK5 and MK10 from MK extract were shown 30.95% and 28.57% of inhibition at 100 mg/kg dose [Table 5] but in case of 50 mg/kg dose it inhibits only 23.80% and 28.57%, respectively ($P < 0.001$ for all). The PG5 and PG10 from PG Linn. extract were shown 35.71% and 45.23% of inhibition at 100 mg/kg dose but in case of 50 mg/kg dose it inhibits only 33.33% and 40.47%, respectively. The F1 and F2 ointment formulation has shown 71.42% and 76.19% of inhibition at 100 mg/kg dose [Table 5] but in case of 50 mg/kg dose it inhibits only 64.28% and 71.42%, respectively. The AH5 and AH10 from AH Lam. extract were shown 50.00% and 57.14% of inhibition at 100 mg/kg dose [Table 5] but in case of 50 mg/kg dose it inhibits only 45.23% and 52.38% [Table 5], respectively. In that case, standard pyrilamine has shown the 88.09% of inhibition at 1 mg/kg dose.

Table 4: Inhibition of carrageenan-induced paw edema in rats at 50 mg/kg and 100 mg/kg dose

Group	Isolated fraction	Dose (mg/kg i.p.)	MEV and SEM	Percent inhibition	Dose (mg/kg i.p.)	MEV and SEM	Percent inhibition
I	Normal	Normal saline	0.42±0.02	----------	Normal saline	0.42±0.02	--------
II	F-1	50	0.11±0.04	73.80	100	0.10±0.04***	76.19
III	F-2	50	0.10±0.03	76.19	100	0.08±0.03***	80.95
IV	F-3	50	0.08±0.03	80.95	100	0.07±0.03	83.33
V	MK5	50	0.28±0.02	33.38	100	0.26±0.02	38.09
VI	MK10	50	0.25±0.02	40.47	100	0.22±0.02	47.61
VII	PG5	50	0.22±0.02	47.61	100	0.21±0.02	50.00
VIII	PG10	50	0.19±0.02	54.76	100	0.18±0.02***	57.14
IX	AH5	50	0.17±0.02	59.52	100	0.15±0.02***	64.28
X	AH10	50	0.15±0.02	64.28	100	0.12±0.02***	71.42
XI	Indomethacin (2.5 mg)		0.05±0.02	88.09	2.5 mg	0.05±0.02	88.09

MEV: Mean edema volume; values represent Mean±SEM; and PI: Percentage inhibition; Group I – (Saline) Edema control; Group II – F-1; Group III – F-2; Group IV – F-3; Group V – MKL5; Group VI – MKL10; Group VII – PGB5; Group VIII – PGB10; Group IX - AHP5; Group X – AHP10; Group XI standard drug indomethacin at the dose of 2.5 mg/kg body weight; *P<0.05; **P<0.01; ***P<0.001, as compared with arthritic control. whereas F1 (*Murraya koenigii* extract (10%) + Ointment base); F2 (*Punica granatum* extract (10%) + Ointment base); F3 (*Artocarpus heterophyllus* extract (10%) + Ointment base), MK: *Murraya koenigii* Linn. extract, PG: *Punica granatum* Linn. extract, AH: Artocarpus heterophyllus Lam. extract

Table 5: Inhibition of paw edema, induced through histamine in rats at 50 mg/kg and 100 mg/kg dose

Group	Isolated fraction	Dose (mg/kg i.p.)	Histamine				Dose (mg/kg i.p.)
			MEV	Percent Inhibition	MEV	Percent inhibition	
I	Normal	Normal saline	0.42±0.02	--------	0.42±0.02	--------	Normal saline
II	F-1	50	0.15±0.04***	64.28	0.12±0.04***	71.42	100
III	F-2	50	0.12±0.03***	71.42	0.10±0.03***	76.19	100
IV	F-3	50	0.09±0.03	78.57	0.08±0.03	80.95	100
V	MK5	50	0.32±0.02	23.80	0.30±0.02	30.95	100
VI	MK10	50	0.30±0.02	28.57	0.29±0.02	28.57	100
VII	PG5	50	0.28±0.02	33.33	0.27±0.02	35.71	100
VIII	PG10	50	0.25±0.02***	40.47	0.23±0.02***	45.23	100
IX	AH5	50	0.23±0.02***	45.23	0.21±0.02***	50.00	100
X	AH10	50	0.20±0.02***	52.38	0.18±0.02***	57.14	100
XI	Pyrilamine	01 mg	0.05±0.02	88.09	0.05±0.02	88.09	1 mg

MEV: Mean edema volume, MK: *Murraya koenigii*, PG: *Punica granatum*, AH: *Artocarpus heterophyllus*. ***P<0.001, as compared with arthritic control.

CONCLUSION

All three plants including AH Lam., MK Linn., and PG Linn. extracted for the active component and have shown the Gallic acid and Quercetin as active constituents for flavonoid and phenolic content and additional used for anti-inflammatory activity of carrageenan- and histamine-induced paw edema in rat. The ointment formulations (F1, F2, and F3) were screened for physicochemical properties. These plant extracts and ointment were carried and evaluated for irritancy test. F3 ointment formulation is better than the F2 and F1 formulation in inhibition and in all phases showing its inhibition of kinins as well as arachidonic acid. MK showed prominent inhibition after the 2 h and their effect gradually decreased showing that their effect was due to inhibition of histamine in the first phase as well as partly due to inhibition of kinins. The order on inhibition in carrageenan induce paw edema is Normal<MKL5<MKL10<PGB5<PGB10<AHP5 <AHP10 <F1 <F2 <F-3 <Standard (Indomethacin). The ointment formulation F3 showed significant inhibition ($P < 0.001$) as compared with standard drug. The order of inhibition in histamine induce paw edema is normal < MKL5 < MKL10 < PGB5 < PGB10 < AHP5 < AHP10 < F1 < F2 < F-3 < Standard (Pyrilamine).

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