SCREENING OF SELECTED MEDICINAL PLANTS FOR ANALGESIC AND ANTI-INFLAMMATORY POTENTIAL

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Abstract: The aim of present paper is to evaluate the analgesic and anti-inflammatory potential of three medicinal plants by using albino rat/mice model. The all three plants i.e. Atrocarpus Heterophyllus (AHEM), Pongamia Glabra (PGEF) and Piper Nigrum (PNEP) has been selected and Soxhlet extraction process is utilized for the separation of active constituents of interest. Albino rat and mice model was employed for the determination of analgesic and anti-inflammatory potential of the plants extract. The plants extract i.e. AHEM, PGEF and PNEP has been tested for the activity having the three different concentration 100, 200 and 400 mg/kg. The result of analgesic activity by the acetic acid induced writhing in mice model, depicted that the plant extract AHEM and PENP at 400 mg/kg concentration has appears to be best effective in reducing the number of wriths by 69.11% and 64.70% respectively, in comparison to acetylsalicylic acid (63.23%). Anti-inflammatory screening by carrageenan induced paw edema rat model suggested that AHEM and PENP at 400mg/kg concentration shown 56.11% and 55.03% inhibition, in contrast to Diclofenac (54.31%). Histopathology of stomach was also performed in cotton pellet induced granuloma model to assess ulcerogenic property and depicted that diclofenac shown more ulceration and congestion in stomach as compare to plants extract. The data obtained from anti-inflammatory and analgesic model suggest that, AHEM having superior analgesic and anti-inflammatory activity than PGEF and PNEP and having potential candidate to cure the inflammation.

Keywords: Anti-inflammatory, analgesic, mice model, piper nigrum, diclofenac.

1. Introduction

Pain remains an important health issue in humans. Pain mechanisms serve as a natural protective function of organisms against noxious stimuli by changing the physiology and behaviour to reduce or avoid further damage, and promote recovery (Benbouzid *et al*., 2008). People with a loss of pain function appear to have recurrent injuries such as burns, repeat fractures, and self-injuries (Ma & Turner 2012).

Pain, an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Melzack *et al.,* 1968). The pain concepts has suggested that thinly myelinated Aδ fibers and unmyelinated C fibers are two types of sensory fibers conducting most of the nociceptive signals to the dorsal horn while the large myelinated Aβ fibers transmit other sensory information to the central nervous system. Pain is external and expressive incident that directly related to the inflammation (Schaible *et al*., 2011). Enhanced production of prostaglandins are directly connected by way of the pain, fever and Inflammation (Yao *et al*., 2016) and these gush cause several disease defects includes arthritis, inflammatory bowel disease and *psoriasis.*

Natural ingredients that extracted from plant resource is the rich sources of terpenes, catechin, alkaloid, glycosides, phenols, anthoxanthin and shown the potential candidates against inflammation and pain. Inflammatory pain is a big health issue causing suffering to millions in both humans and animals especially chronic inflammation such as arthritis and inflammatory bowel disease. Unlike pain originated from acute inflammation that act as a physiological function to prevent further damage and cease after the noxious stimulus is removed, chronic inflammation pain occurs when healing persists beyond the expected time, due to ongoing of inflammatory process (Ossipov *et al*., 2010).

The model has helped scientists understand the underlying mechanism of inflammatory pain and develop potential treatments. To induce inflammation, irritating substances or the inflammatory mediator is injected into the body part of an animal such as the hindpaws. Formalin, carrageenan, capsaicin, and complete Freund's adjuvant (CFA) are common inflammatory substances that can irritate tissue and provoke inflammatory responses (Vierck *et al*., 2008).

Natural chemical agents extracted from plants that can modulate the expression of pro-inflammatory signals clearly have potential against pain and inflammation. These include flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins, polyphenols and anthoxanthins, all of which are known to have anti-inflammatory effects. The phytoconstituents which have been attributed with anti-inflammatory activity are triterpenoid and their glycosides includes aescin (*β*-amyrin), chiisanosides (lupine triterpenoids), dysobinin, boswellic acid and pentacyclic triterpenoid acids, *α*-amyrin & Taxifolin, Sorghumol, Bassic acid (Juteaua *et al.,*2002).

In Asian countries, 60-70% patient living in rural areas are dependent on herbal medicine for their day to day disease (Singh *et al*., 2009). Number of diseases is cured by herbal drugs. The pain and inflammation also cured by herbal medicines. Various drugs obtained from plant sources may produce their anti-inflammatory actions by various proposed mechanisms i.e. Inhibition of cycloxygenase enzyme; Inhibition of Leucocyte migration to the site of inflammation; Inhibition of Arachidonic acid synthesis and Inhibition of release of histamine from mast cells.

The aim of the presented paper was to evaluated the analgesic and anti-inflammatory potential of three medicinal plants i.e.. Atrocarpus Heterophyllus (AHEM), Pongamia Glabra (PGEF) and Piper Nigrum (PNEP). The plants extract as AHEM, PGEF and PENP, was used at different concentration (100, 200 and 400 mg/kg), to assess the analgesic and anti-inflammatory activity by using the analgesic albino mice model as well as anti-inflammatory based carrageenan induced albino rat model.

2. Material and Methods:

Drugs are collected from wild or cultivated plants. The season in which the drug is collected having an important role to the quality of drug. Generally, three methods are employed in the extraction of plant materials as Maceration, Percolation, Soxhlet extraction. Soxhlet extraction is rapid and may be employed in extraction of sparingly soluble constituents which cannot be done by either maceration or percolation methods.

2.1 Plant Material collection and authentication

The fruits of Atrocarpus heterophyllus Linn were collected at in the month of july, 2016 from local field areas of Bhopal region, M.P., leaves of Pongamiaglabra from Garden and fruits of Piper nigrumfrom local market of Bhopal, Madhya Pradesh. The specimens were submitted and identified as fruits of *Atrocarpus heterophyllus* Linn (AHEM) family of Moraceae, leaves of Pongamia glabra (PGEF) family Fabaceae and fruits of Piper nigrum (PNEP) family of Pipereaceae and authenticated by Dr.Zia ul Hassan of the Department of Botany, Saifia Science College, Bhopal. The appession no. for the specimen is 490/BS/saifia/16 has been preserved for future identification. The samples were shade dried so as to protect its chemical constituents not to get degrade at high temp.

2. 2 Physicochemical characterization

These characterization parameters give the idea of the physical characteristics and the chemical correlation of constituents with it, present in the herbal drugs. They involve the determination of ash values, foreign matter, extractable matter and volatile oil content of the preparations or individual drugs (Ansari *et al*., 2006).

2.2.1 Determination of foreign matter:

Hundred grams of the sample was weighed and spread on a white tile uniformly without overlapping. Then the sample was inspected by means of 3x lens and the foreign organic matter was separated. After complete separation the matter was weighed and percentage w/w was determined (Kadam *et al*., 2012).

2.2.2 Determination of solvent extractive values:

2.2.2.1 Determination of water soluble extractive value:

Five grams of powdered drug was macerated with 100 ml of water closed flask for 24hr and was occasionally shaked with 6hr time period and was allowed to stand for 18hr. After filtration the 23ml of the filterate evaporated to dryness in a tared flat bottomed shallow dish. Dry at 103^{o}C and weighed. Percentage of water soluble extractive value was calculated with reference to the air dried drug.

2.2.2.2 Determination of alcohol soluble extractive value:

Five grams of powdered drug was macerated with 100ml of ethanol closed flask for 24hr and was occasionally shaked with 6hr time period and was allowed to stand for 18hr. After filtration the 23ml of the filtrate evaporated to dryness in a tared flat bottomed shallow dish. Dry at 103^{o}C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to the air dried drug.

2.2.3 Determination of ash value:

2.2.3.1 Determination of total ash:

Total ash was determined by weighing 2-3gm of the air dried crude drug in the tared platinum or silica dish and incinerated at a temperature not exceeding 430^{o}C until free from carbon and then was cooled and weighed.

2.2.3.2 Determination of acid insoluble ash:

Ash insoluble in HCI is the residue obtained after extracting the sulfated or total ash with HCI and calculated with reference to 100gm of drug. The ash obtained from the previous process was boiled with 23ml of 2M HCI for 3min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a dessicator and weighed. Percentage of acid insoluble ash was calculated with reference to the air dried drug.

2.2.3.3 Determination of water soluble ash:

The ash was boiled with 23ml of water for 3 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited for 13min. at a temperature not exceeding 43^{o}C. The weight of the insoluble matter was substracted from the weight of the ash and this represents the water soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.

2.3 Extraction

The leaves of NI, pulp of AH, bark of PG and leaves of MK were separated from the fresh and dried on filter paper sheets under shade at room temperature until with changing of color of filter papers. The shade-dried, coarsely powdered materials (300g) were defatted by petroleum ether (43^{0}C). The defatted marc was then subjected to soxhlet extraction (Fig 3.1) with 70% ethanol to obtain hydroalcoholic extract. The hydroalcoholic extracts were evaporated under reduced pressure at low temperature (30^{o}C) to dryness to yield different extracts, stored in an airtight container in refrigerator for further experimental studies.

2.4 Qualitative test analysis

In order to detect the various constituents present in the different extracts, those were subjected to the tests (Ansari *et al*., 2006; Jarald *et al*., 2007; Chopade *et al*., 2008). Phyto-chemical screening was performed for the detection of Alkaloids, Glycosides, Carbohydrates, Tannins, Resins, Flavanoid, Steroids, Proteins and Amino acids.

2.5 Pharmacological evaluation of plants extract

2.5.1 Experimental animals

Female Swiss albino mice (25-30 g) and Female Wistar rats (180–220 g) were purchased from National Institute of Biosciences, Pune, India. Animals were housed in an air-conditioned room at a temperature of 25±1°C and relative humidity of 45% to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellet (Manufactured by Pranav Agro Industries Ltd., Sangli, India) and water ad libitum.

2.5.2 Research protocol approvals

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India (Protocol approval number was **(Reg. No. 780/PO/Re/S/03/ CPCSEA) [RKDFCP/IAEC/2019/25]**.

2.5.3 Acute oral toxicity study

Healthy female Swiss albino mice of 25-30 g were used in acute toxicity studies as per OECD guidelines-425. The animals were fasted overnight and divided into 3 groups with 5 mice in each group. Extracts (PNEP, AHEM, PGEF) were administered at dose of 2000 mg/kg, p.o. body weight. The mice were observed continuously for behavioural and autonomic profiles for 2 hrs and for any signs of toxicity or mortality up to 48 hrs (OECD-425, 2001).

2.6 Analgesic activity

2.6.1 Hot plate test in mice

Female Swiss albino mice (25–30 g) were treated according to the method described by Eddy and Leimback, 1953. Mice were screened by placing them on hot plate (UGO Basile, Italy. Model No. DS-37) maintained at 55±1°C and the reaction time was recorded in seconds. The time for paw licking or jumping on the hot plate was considered as a reaction time. The responses were recorded before and after 30,60,90,120,150 and 180 min of the administration of PNEP, AHEM, PGEF and pentazocine. A cut-off time of 15s was used to avoid injury to the animals. The mice were divided into eleven groups with six mice in each group.

Group I: - Vehicle control (2% Tween 80).

Group II: - Standard (Pentazocine 5 mg/kg, s.c.).

Group III, IV and V: - PNEP (100, 200 and 400 mg/kg, p.o.), respectively.

Group VI, VII and VIII: - AHEM* BEST (100, 200 and 400 mg/kg, p.o.), respectively.

Group IX, X and XI: - PGEF (100, 200 and 400 mg/kg, p.o.), respectively.

2.6.2 Acetic acid induced writhing in mice

Female Swiss albino mice (25–30g) were treated according to the method described by Collier ***et al***, 1963. Mice were pre-treated orally with PNEP, AHEM, PGEF and acetylsalicylic acid, 60 min before administration of acetic acid solution at a dose of 10 ml/kg (0.6%, i.p.). The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 15 min.

The mice were divided into eleven groups of six mice each.

Group I: - Vehicle control (2% Tween 80).

Group II: - Standard (Acetylsalicylic acid 100 mg/kg p.o.).

Group III, IV and V: - PNEP (100, 200 and 400 mg/kg, p.o.), respectively.

Group VI, VII and VIII: - AHEM (100, 200 and 400 mg/kg, p.o.), respectively.

Group IX, X, and XI: - PGEF(100, 200 and 400 mg/kg, p.o.), respectively.

The percent inhibition of writhing was calculated as follows:

% Inhibition = (VC-VT/VC) * 100***;*** Where, VT, number of writhes in drug treated mice; VC, number of writhes in control group mice.

2.7 Anti-inflammatory activity

The various extracts and fractions were subjected to pharmacological screening to identify fractions responsible for anti-inflammatory activity. Screening was done by using various models to identify the mechanism of action (Amer *et al*., 2012).

2.7.1 Carrageenan induced paw edema in rats

Female Wistar rats (180–220 g) were treated according to the method described by Winter ***et al***, 1962. Inflammation was produced by injecting 0.1ml of 1% lambda carrageenan in sterile normal saline into the sub plantar region of the right hind paw of the rat. Rats were pre-treated orally with PNEP, AHEM, PGEF and diclofenac 1h before the carrageenan injection. The paw volume was measured from 0-6 h, at an hourly interval using plethysmometer (Ugo Basile, Italy, Model No. 7140). The mean changes in injected paw volume with respect to initial paw volume were calculated.

Female Wistar rats were divided into eleven groups of six rats each.

Group I: - Carrageenan control (2% Tween 80).

Group II: - Standard (Diclofenac 10 mg/kg p.o.).

Group III, IV and V: - PNEP (100, 200 and 400 mg/kg, p.o.), respectively.

Group VI, VII and VIII: - AHEM (100, 200 and 400 mg/kg, p.o.), respectively.

Group IX, X and XI: - PGEF (100, 200 and 400 mg/kg, p.o.), respectively.

Percentage inhibition of paw volume between treated and control group was calculated by the following formula, % Inhibition = (VC-VT / VC *100).

Where, VT and VC are the mean increase in paw volume in treated and control groups, respectively.

2.7.2 Cotton pellet induced granuloma in rats

Method described by D'Arcy *et al*, 1960 was followed. Chronic inflammation was produced by implanting the pre-weighed sterile cotton pellets (50 mg) in the axilla region of the each rat through a small incision. PNEP, AHEM, PGEF and diclofenac were administered orally for seven consecutive days after the cotton pellet implantation. Before implanting the cotton pellets, rats were anaesthetized with anaesthetic ether. On the eight day animals were sacrificed by cervical dislocation and stomach was removed for histopathology study and cotton pellets were removed from animal's body, freed from the extraneous tissues, dried in oven at 60 °C for 24 h and weighed.

Female Wistar rats weighing (180 – 220 g) were divided into eleven groups of six rats each.

Group I: - Vehicle control (2% Tween 80).

Group II: - Standard (Diclofenac 10 mg/kg p.o.).

Group III, IV and V: - PNEP (100, 200 and 400 mg/kg, p.o.), respectively.

Group VI, VII and VIII: - AHEM (100, 200 and 400 mg/kg, p.o.), respectively.

Group IX, X and XI: - PGEF (100, 200 and 400 mg/kg, p.o.), respectively.

3. RESULT AND DISCUSSION

3.1 Physicochemical properties

Temperature and types of solvent can give impact on the quantity of extractable matter of a plant. The extractive capacity (measured as extractive value) increases with the amount of extractive matter produced under a particular condition. The herbal monograph specified that the limits for water soluble extractive values are not less than 3% and 2%, respectively for water and alcohol soluble extractive values. All specimens were found superior than the standard specifications. From this study, higher temperature and using water as solvent exhibited a better extractive capacity than in room temperature and alcohol based solvent.

Table 1: Solvent Extractive Values of Crude Drugs

S. NO	Name of the drug	Water soluble extractive value (% w/w)		Alcohol soluble extractive value (% w/w)	
		Theoretical	Obtained	Theoretical	Obtained
1.	*Artocarpus heterophyllus (*AHEM*)*	>10	20.9±0.53	>02	12.86±0.12
2.	*Pongamia glabra* (PGEF)	>09	12.90±0.24	>06	9.31±0.25
3.	*Piper nigrum (*PNEP)	>03	9.14±0.06	>02	11.2±0.08

The water soluble extractive value indicated the presence of sugar, acids and inorganic compounds; the water soluble extractive value found to be 09.14±0.06, 20.90±0.53 and 12.90±0.24 %w/w for PNEP, AHEM and PGEF respectively **(Table 1)**. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. The alcohol soluble extractive value was found to be 12.86±0.12, 9.31±0.25 and 11.2±0.08 (%w/w) for AHEM, PGEF and PNEP which signify the nature of the phyto-constituents present in plant.

Table 2: Physical characteristics of PNEP, AHEM, PGEF Extract

S. NO	Name of the Drug	Values	Foreign organic matter	Total Ash value	Acid insoluble ash value	water soluble ash value
1.	*Pongamia glabra (PGEF)*	Theoretical	<2%	<5%	<1%	-
		Observed	0.48±0.072%	3.87±0.084%	0.71±0.037%	2.84±0.071%
2.	*Artrocarpus heterophyllus (*AHEM*)*	Theoretical	<1%	<0.5%	<0.2%	-
		Observed	0.36±0.002%	0.25±0.031%	0.05±0.007%	0.07±0.001%
3.	*Piper nigrum (*PNEP*)*	Theoretical	<2%	<3%	<2%	-
		Observed	1.25±0.024%	1.79±0.044%	1.32±0.084%	0.87±0.077%

Evaluation of crude drug ensures the identity of drug and determines the quality and purity of drugs. The main reason behind the need for the evaluation of crude drug is biochemical variation in the drug, effect of treatment, storage of drug, adulteration and substitution. The results of the physicochemical parameters of fruit powder lie within the limit which is mentioned in **Table 2.** The results of foreign organic matter denote presence of any organism, part or product of an organism, other than that

named in the specification and description of the herbal material concerned, which was found to be for PGEF (0.48±0.072%), AHEM (0.36±0.002%) and PNEP (1.25±0.024%). A high Ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing. All the individual drugs were found to have total Ash values in the range from 0.25 to 3.87% w/w **(Table 2).** The PGEF, AHEM and PNEP have 3.87±0.084, 0.25±0.031% and 1.79±0.044% w/w total Ash values**.** The water-soluble ash values of the individual drugs were in the range of 0.07 to 2.84% w/w. This shows a normal quality of the drugs. Water-soluble ash values of the PGEF, AHEM and PNEP were found to be in the range of 0.07 to 2.84 (% w/w)**.** The acid-insoluble ash values of the individual drugs ranges from 0.05 to 1.32 and is below 2.0% for all the drugs **(Table 2).** The ash value determinations are important parameter to standardize the herbal drugs.

3.2 Extraction

The extraction was done by successive solvent extraction, to increase the extraction, to achieve separation of compounds in different extracts and decrease the time taken by extraction process the flask and soxhlet apparatus was covered by cotton to increase the insulation. The drying of extract containing solvent (75% ethanol, hydro-alcoholic solution) was done by vacuum distillation process.

3.3 Qualitative analysis

Preliminary Phytochemical screening was performed for extracts of Artocarpus heterophyllus (AHEM), Pongamia glabra (PGEF) and Piper nigrum (PNEP). It was noted that extracts of AHEM contains flavonoids, glycosides, alkaloids, tannins, carbohydrates, saponins, steroids, fats, oils, protein and amino acids. The flavanoidal content test for qualitative analysis was given with large intensity for alcoholic extract of Artocarpus heterophyllus*.* The extracts of PGEF contain flavonoids, glycosides alkaloids, tannins, carbohydrates, , saponins, steroids, fats, oils, protein and amino acids. The extracts of PNEP contain alkaloids, flavanoids, tannins, carbohydrates, glycosides, steroids, fats, oils, protein and amino acids **(Table 3)**.

Table 3: Qualitative analysis of hydro-alcoholic extract of PNEP, AHEM and PGEF

S. No	Test	PENP	AHEM	PGEF
1	**Alkaloids**			
a.	Dragendorff's test	+ve	+++ve	++ve
b.	Hager's test	+ve	+++ve	+++ve
c.	Wagner's test	+ve	++ve	+ve
d.	Mayer's test	+ve	+++ve	++ve
2	**Tannins**			
a.	Vanillin-HCl test	+ve	+ve	+ve
b.	Ferric chloride test	+ve	+ve	+ve
c.	Gelatin test	+ve	+ve	+ve
3	**Carbohydrates**			
a.	Molish test	+ve	-ve	+ve
b.	Fehling test	+ve	-ve	+ve
b.	Benedict test	+ve	-ve	+ve
4	**Glycoside**			
a.	Keller Killani	+ve	+ve	-ve
b.	Legal test	+ve	+ve	-ve
c.	Borntrager test	+ve	+ve	-ve
5	**SAPONINS**			
a.	Foam test	+ve	-ve	-ve
6	**FLAVONOIDS**			
a.	Shinoda test	+ve	++ve	++ve
b.	Lead Acetate test	+ve	++ve	+ve

7	**STEROIDS**			
a.	LibermannBurchard test	+ve	-ve	-ve
b.	Salkowski Reaction	+ve	-ve	-ve
8	**FATS & OILS**			
a.	Filter paper Test	+ve	-ve	-ve
b.	Dye Test	++ve	-ve	-ve
9	**PROTEINS AND AMINO ACIDS**			
a.	Millions Test	+ve	+ve	+ve
b.	Biuret Test	+ve	+ve	+ve
c.	Precipitation Test	+ve	-ve	-ve
d.	Ninhydrin Test	+ve	++ve	++ve

Where AHEM: Hydro-alcoholic extract of Artocarpus heterophyllus; PGEF: Hydro-alcoholic extract of Pongamia glabra; PNEP: Hydro-alcoholic extract of Piper nigrum '-' means negative result, '+' means positive results, '++' or '+++' means intensity of reesult

3.4 Pharmacological assessment

3.4.1 Acute toxicity test

Administration of 2000 mg/kg, p.o. of all the three extracts PNEP, AHEM and PGEF did not produce any behavioral abnormalities and mortality (Table 4). So the dose selected for further study was 100, 200 and 400 mg/kg, p.o. for each extracts.

Table 4: Acute toxicity test of PNEP, AHEM and PGEF extract

S. No.	Extracts 2000 mg/kg, p.o.	No. of animals dead/survived
1.	PNEP	0/5
2.	AHEM	0/5
3.	PGEF	0/5

3.5 Analgesic activity

3.5.1 Effect of oral administration of PNEP, AHEM, and PGEF extract on hot plate test in mice

In hot plate test, pentazocine (5 mg/kg, s.c.) significantly ($p<0.001$) increased the paw withdrawal latency at 60 and 90 minutes. Onset of action was observed at 60 minutes of administration of pentazocine. However, the extracts PNEP at 400 mg/kg concentration inhibit the pain, AHEM at 200 and 400 mg/kg dose inhibit the pain sensation and PGEF at 400 mg/kg concentration inhibit pain produced by thermal means **(Table 5)**.

Table 5: Effect of oral administration of PNEP, AHEM, and PGEF extract on hot plate test in mice

Treatment Groups	Paw withdrawal latency (Sec)						
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
Vehicle Control	6.02 ± 0.45	5.45 ± 0.44	5.50 ± 0.62	4.83 ± 0.21	5.65 ± 0.55	5.83 ± 0.41	5.68 ± 0.58
Pentazocine (5 mg/kg)	5.65 ± 0.50	5.90 ± 0.38	9.15 ± 0.50***	11.33 ± 0.36***	7.98 ± 0.40**	6.08 ± 0.58	5.87 ± 0.40
PNEP (100 mg/kg)	5.95 ± 0.58	5.03 ± 0.38	4.63 ± 0.67	6.10 ± 0.49*	4.35 ± 0.21	5.27 ± 0.58	5.10 ± 0.35
PNEP (200 mg/kg)	4.73 ± 0.53	5.58 ± 0.57	4.60 ± 0.49	5.30 ± 0.24	5.77 ± 0.58	5.82 ± 0.38	5.78 ± 0.21
PNEP (400 mg/kg)	6.50 ± 0.40	4.98 ± 0.51	7.63 ± 0.46	9.15 ± 0.51**	5.92 ± 0.32	5.55 ± 0.37	5.22 ± 0.60
AHEM (100 mg/kg)	4.90 ± 0.39	5.43 ± 0.54	5.86 ± 0.39	7.45 ± 0.44*	5.38 ± 0.27	5.80 ± 0.38	5.97 ± 0.69
AHEM	5.22 ± 0.38	5.35 ±	6.96 ±	8.43 ±	6.40 ±	5.20 ±	5.27 ±

(200 mg/kg)		0.40	0.49	0.51**	0.47	0.30	0.54
AHEM (400 mg/kg)	5.38 ± 0.43	5.85 ± 0.45	8.65 ± 0.36	10.45 ± 0.57**	6.62 ± 0.57	5.17 ± 0.33	5.72 ± 0.47
PGEF (100 mg/kg)	5.18 ± 0.58	4.55 ± 0.53	5.02 ± 0.34	5.65 ± 0.37	5.28 ± 0.60	5.55 ± 0.26	5.60 ± 0.36
PGEF (200 mg/kg)	5.30 ± 0.40	5.55 ± 0.30	6.02 ± 0.34	5.32 ± 0.26	6.02 ± 0.47	5.17 ± 0.30	5.33 ± 0.41
PGEF (400 mg/kg)	5.62 ± 0.44	5.32 ± 0.40	6.26 ± 0.25	7.15 ± 0.30**	5.68 ± 0.45	5.30 ± 0.23	5.55 ± 0.51

Values are expressed as mean ± S.E.M.; n=6 mice per group. Two way ANOVA followed by Bonferroni post hoc test when compared with vehicle control

**p<0.01, ***p<0.001.

3.5.2 Effect of oral administration of PNEP, AHEM, and PGEF extract on acetic acid induced writhing in mice

AHEM(200 and 400 mg/kg) significantly (p<0.05 and p<0.001, respectively) reduced the number of writhes induced by 0.6% acetic acid at the dose of 100 mg/kg. Also PNEP(400 mg/kg) and PGEF (400 mg/kg) showed a significant (p<0.05) reduction in number of writhes when compared to vehicle control group. While (100 mg/kg), PNEP (100 and 200 mg/kg) and PGEF (100, 200 mg/kg) showed non-significant reduction in writhing. The number of writhes in the acetic acid vehicle control group was found to be 68±1.5. Acetylsalicylic acid (100 mg/kg) appears to be better effective in reducing the number of writhes, it significantly (p<0.001) reduced the number of writhes by 63.23% but the extract AHEM (200 mg/kg) and AHEM (400 mg/kg), appears to be best effective in reducing the number of writhes, it significantly (p<0.001) has shown the reduction in writhes by 52.94 and 69.11% respectively **(Table 6)**. In other cases, extract, PNEP (400 mg/kg) appears to be good effective in reducing the number of writhes by 64.70%, it significantly (p<0.05) **(Fig 1).**

Table 6: Effect of oral administration of PNEP, AHEM, and PGEF extract on acetic acid induced writhing in mice

Group No.	Treatment groups	Number of writhing	Percentage inhibition
I	Vehicle control	68 ± 1.5	-
II	Acetyl salicylic acid (100 mg/kg)	25 ± 2.1***	63.23
III	PNEP (100 mg/kg)	52 ± 2.0 *	23.52
IV	PNEP (200 mg/kg)	44 ± 3.7 **	35.29
V	PNEP (400 mg/kg)	24 ± 3.4***	64.70
VI	AHEM (100 mg/kg)	42 ± 2.6 **	38.23
VII	AHEM (200 mg/kg)	32 ± 2.2**	52.94
VIII	AHEM (400 mg/kg)	21 ± 3.3***	69.11
IX	PGEF (100 mg/kg)	54 ± 2.0 *	20.58
X	PGEF (200 mg/kg)	48± 2.5 *	29.41
XI	PGEF (400 mg/kg)	38 ± 3.0 **	44.11

Values are expressed as mean ± S.E.M.; n=6 mice per group. One way ANOVA followed by Dunnett's test when compared with vehicle control *p<0.05, ***p<0.001.

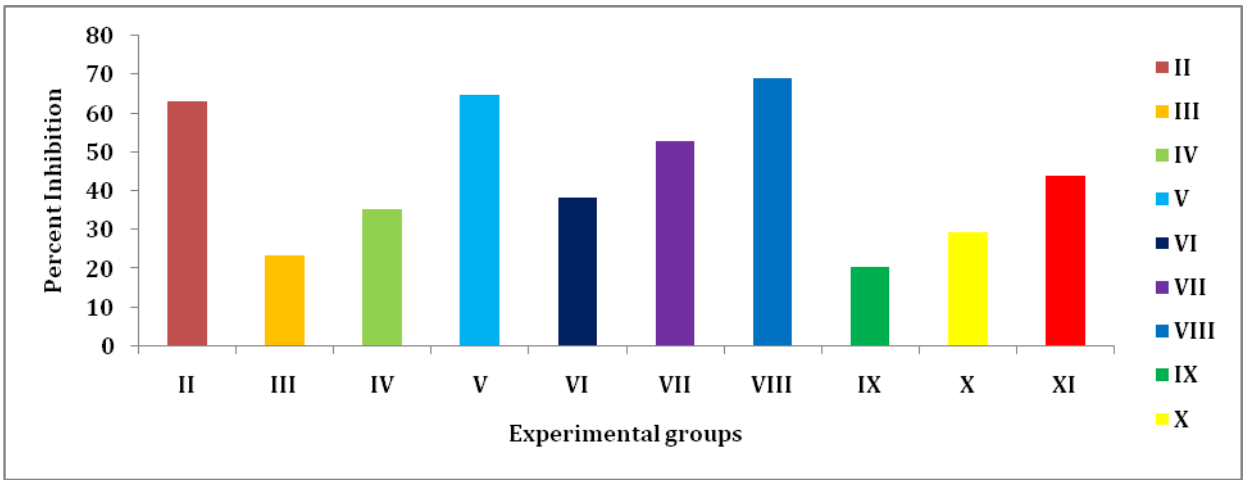


Fig 1: Effect of oral administration of PNEP, AHEM, and PGEF extract on acetic acid induced writhing in mice

3.6 Anti-inflammatory activity

3.6.1 Effect of oral administration of PNEP, AHEM, and PGEF extract on carrageenan induced paw edemain rats

There was a gradual increase in paw volume of rats in the carrageenan control group. In the test groups, the AHEM (200 and 400 mg/kg) showed a significant (p<0.001) reduction in paw volume in a dose dependent manner at 3rd and 5th h. The inhibitory effect of the AHEM at (400 mg/kg) was found to be 49.21% at 3h and 56.11% at 5h. However, PNEP (400 mg/kg) showed significant (p<0.001) inhibition in paw volume at 5h with 55.03% inhibition when compared to carrageenan control group (Fig 2). Diclofenac (10 mg/kg) caused significant (p<0.001) inhibition of increase in paw volume at 3rd and 5h. The inhibitory effect of the diclofenac at 10 mg/kg was 46.48% at 3h and 54.31% at 5h (Table 7).

Table 7: Effect of oral administration of PNEP, AHEM, and PGEF extract on carrageenan induced paw edema in rats

Experiment Group No.	Treatment	Change in paw volume (ml)		
		1 h	3 h	5 h
I	Carrageenan control	1.43 ± 0.24	2.56 ± 0.10	2.78 ± 0.07
II	Diclofenac (10 mg/kg)	1.10 ± 0.11 (23.07)	1.37 ± 0.04*** (46.48)	1.27 ± 0.04*** (54.31)
III	PNEP (100 mg/kg)	1.35 ± 0.04 (5.59)	1.65 ± 0.11 (35.54)	1.68 ± 0.11 (39.56)
IV	PNEP (200 mg/kg)	1.23 ± 0.04 (13.98)	1.53 ± 0.12 (40.23)	1.52 ± 0.12* (45.32)
V	PNEP (400 mg/kg)	1.08 ± 0.05 (24.47)	1.34 ± 0.08* (47.65)	1.25 ± 0.07*** (55.03)
VI	AHEM(100 mg/kg)	1.22 ± 0.06 (14.68)	1.48 ± 0.10* (42.18)	1.42 ± 0.09** (48.92)
VII	AHEM(200 mg/kg)	1.15 ± 0.05 (19.58)	1.40 ± 0.09*** (45.31)	1.33 ± 0.07*** (52.15)
VIII	AHEM(400 mg/kg)	1.05 ± 0.06 (26.57)	1.30 ± 0.04*** (49.21)	1.22 ± 0.05*** (56.11)
IX	PGEF (100 mg/kg)	1.37 ± 0.16 (04.19)	1.78 ± 0.18 (30.46)	1.75 ± 0.17 (37.05)
X	PGEF (200 mg/kg)	1.28 ± 0.09	1.59 ± 0.11 (37.89)	1.58 ± 0.12 (43.16)

		(10.48)		
XI	PGEF (400 mg/kg)	1.20 ± 0.04 (16.08)	1.45 ± 0.12** (43.35)	1.38 ± 0.13** (50.35)

Values are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni post hoc test when compared with carrageenan control *p<0.05, **p<0.01, ***p<0.001. The Figs in parenthesis indicate the percent inhibition.

Table 8: Percent inhibition on inflammation by oral administration of PNEP, AHEM, and PGEF extract on carrageenan induced paw edema in rats

Experiment Groups	Treatment	Percent inhibition		
Group No.		1 h	3 h	5 h
I	Carrageenan control	-	-	-
II	Diclofenac (10mg/kg)	23.07	46.48	54.31
III	PNEP (100 mg/kg)	5.59	35.54	39.56
IV	PNEP (200 mg/kg)	13.98	40.23	45.32
V	PNEP (400 mg/kg)	24.47	47.65	55.03
VI	AHEM(100 mg/kg)	14.68	42.18	48.92
VII	AHEM(200 mg/kg)	19.58	45.31	52.15
VIII	AHEM(400 mg/kg)	26.57	49.21	56.11
IX	PGEF (100 mg/kg)	04.19	30.46	37.05
X	PGEF (200 mg/kg)	10.48	37.89	43.16
XI	PGEF (400 mg/kg)	16.08	43.35	50.35

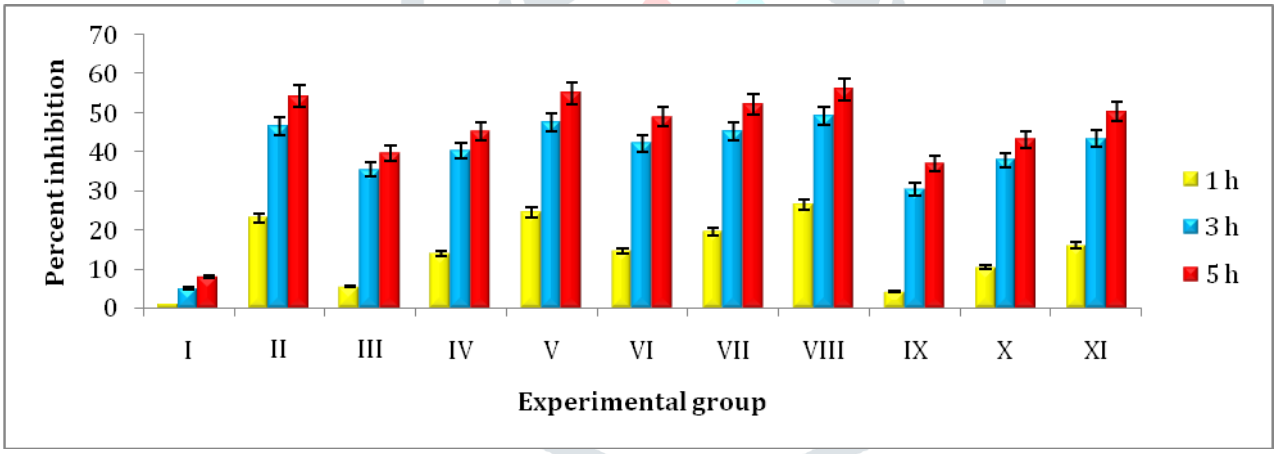


Fig 2: Percent inhibition by oral administration of PNEP, AHEM and PGEF extracts on carrageenan induced paw edema in rats

3.6.2 Effect of oral administration of PNEP, AHEM, and PGEF extract on cotton pellet induced granuloma in rats

AHEM (200 and 400 mg/kg) significantly (p<0.001) inhibited the granuloma formation in a dose dependent manner with (58.82% and 74.11% inhibition, respectively), when compared to vehicle control group. PNEP (400 mg/kg) also significantly (p<0.01) inhibited the granuloma formation with 70.58% inhibition (Fig 3). However there was no significant inhibition in granuloma formation on treatment with PGEF at all the doses tested. Diclofenac (10 mg/kg) also significantly (p<0.001) inhibited granuloma formation with maximum inhibition of 67.05% **(Table 9, Fig 3)**.

Table 9: Effect of oral administration of PNEP, AHEM, and PGEF extract. on cotton pellet induced granuloma in rats

Experiment Groups	Treatment groups	Increase in weight of cotton pellet (mg)	Percent inhibition
I	Vehicle Control	85 ± 3.1	-

II	**Diclofenac (10 mg/kg)**	28 ± 1.2***	67.05
III	**PNEP (100 mg/kg)**	58 ± 3.5	31.74
IV	**PNEP (200 mg/kg)**	49 ± 1.7	42.35
V	**PNEP (400 mg/kg)**	25 ± 2.5**	70.58
VI	**AHEM(100 mg/kg)**	44 ± 2.1**	48.23
VII	**AHEM(200 mg/kg)**	35 ± 2.8***	58.82
VIII	**AHEM(400 mg/kg)**	22 ± 2.0***	74.11
IX	**PGEF (100 mg/kg)**	62 ± 2.5	27.05
X	**PGEF (200 mg/kg)**	54 ± 2.4	36.47
XI	**PGEF (400 mg/kg)**	40 ± 3.1**	52.94

Values are expressed as mean ± S.E.M.; n=6 rats per group. One way ANOVA followed by Dunnett's test when compared with vehicle control **p<0.01, ***p<0.001.

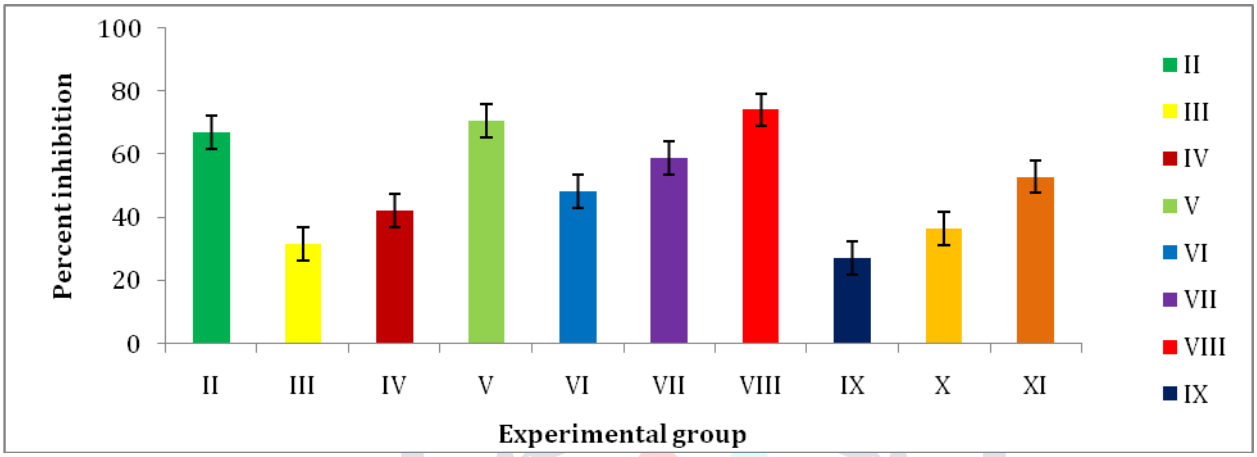
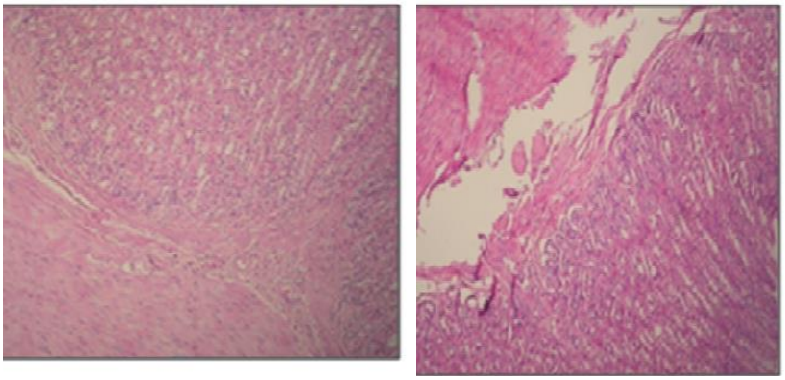


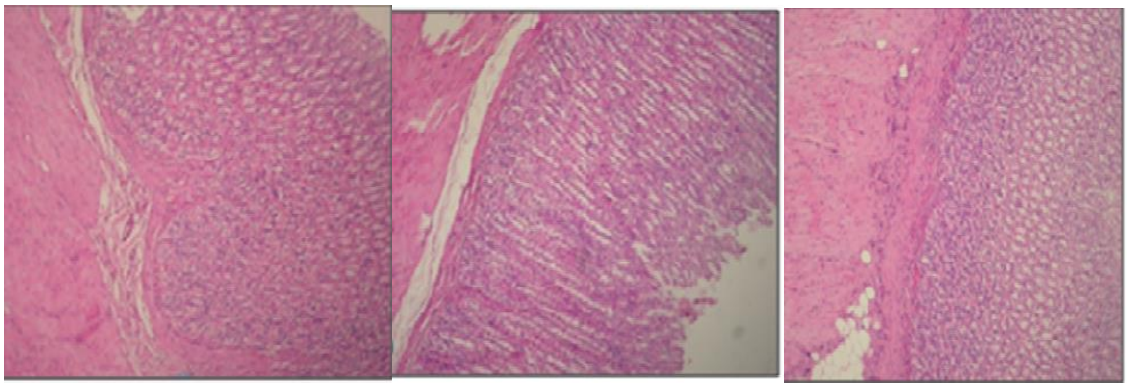
Fig 3: Percent inhibition by oral administration of PNEP, AHEM and PGEF extracts on cotton pellet induced granuloma in rats

3.6.3 Gastric ulcerogenic effect of oral administration of PNEP, AHEM, and PGEF extract in cotton pellet induced granuloma in rats

Histopathology of stomach of vehicle control group rats showed intact gastric mucosa, with no ulceration and no congestion. All the rats treated with (PNEP, AHEM and PGEF) at dose of (400 mg/kg) showed less ulcer and absence of congestion when compared to the standard group treated with diclofenac. Diclofenac (10 mg/kg) treated rats showed ulceration and congestion **(Fig 4)**.



A. Vehicle control **B. Diclofenac 10 mg/kg**



C. AHEM-200 **D. PNEP-400** **E. AHEM-400**

Fig 4: Histopathology of stomach in cotton pellet induced granuloma in rats

3.7 DISCUSSION

In the present investigation the fruits of *Atrocarpus heterophyllus* (AHEM) family of Moraceae, leaves of Pongamia glabra (PGEF) family Fabaceae and fruits of Piper nigrum (PNEP) family of Pipereaceae was selected for evaluation of analgesic and anti-inflammatory activity. Three plant extracts with 100, 200 and 400mg/kg concentration were prepared and label as AHEM, PGEF and PNEP. Phytochemical analysis of the extracts revealed presence of flavonoids, alkaloids, phenols, tannins in AHEM, PGEF and PNEP along with saponins, steroids, and triterpenoids. Glycosides, proteins, carbohydrate and amino acids were found to be present in AECP. Acute oral toxicity studies performed according to OECD guideline- 425 revealed that all the three extracts were safe at the dose of 2000 mg/kg. Analgesic activity of the three extracts was investigated using hot-plate test and acetic acid induced writhing model in Swiss albino mice. In hot plate test, plants extract (AHEM, PENP and PGEF) at 400 mg/kg concentration has shown increase in pain latency, while standard drug pentazocine showed significant increase in pain latency. In acetic acid induced writhing model, AHEM (200, 400 mg/kg) showed significant decrease in number of writhings than PGEF (100, 200 and 400 mg/kg) and PNEP (100, 200 and 400 mg/kg).Results of analgesic activity suggest that, the extract AHEM (200 and 400 mg/kg) has highest peripheral analgesic potential than PGEF (100, 200 and 400 mg/kg) and PNEP (100, 200 and 400 mg/kg). Anti-inflammatory activity of all the three extracts were investigated using carrageenan induced rat paw edema model and cotton pellet induced granuloma model in Wistar rats. In carrageenan induced rat paw edema model, AHEM (200 and 400 mg/kg) dose dependently showed significant decrease in paw volume.

PGEF(100 and 200 mg/kg) and PNEP (100 and 200mg/kg) was found to be less active in decreasing paw volume. In cotton pellet induced granuloma model, AHEM (200 and 400mg/kg) showed significant inhibition of granuloma formation as compare to PGEF (100 and 200mg/kg) and PNEP (100 and 200mg/kg). Histopathology of stomach was also performed in cotton pellet induced granuloma model to assess ulcerogenic property of plants extract and standard drug diclofenac. Diclofenac showed ulceration and congestion in stomach. In comparison to that all the three extracts showed lesser ulceration and congestion. Results of anti-inflammatory activity in carrageenan-induced rat paw edema model as well as cotton pellet induced granuloma model suggested that, AHEM had good anti-inflammatory activity than PGEF and PNEP. AHEM showed superior analgesic and anti-inflammatory activity than PGEF and PNEP.

3.8 ACKNOWLEDGMENT

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3.9 CONFLICTS OF INTEREST

The authors has on conflict of interest.

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