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Evaluation of Analgesic and Anti-inflammatory activity study of Polyherbal formulation ACUPEN in experimental animal models

Research Article

Atul Desai[1*], Hemshree Desai[2], Rutvij Desai[3], Rohit Yadav[4], Chirag Desai[4], Merai Ankit[4], Arindam Paul[4]

1. Ayurveda Physician, 2. Modern Medicine Practitioner,
Dhanvantari Clinic, Ayurveda Health Care and Research Centre, Vyara, Gujarat, India.
3. Master of Science; Manila Central University, Philippines.
4. Department of Pharmacology: ROFEL Shri G.M. Bilakhia College of Pharmacy, RSRU, Vapi. India.

Abstract

Aim and Objective: Evaluation of Analgesic and Anti-Inflammatory activity study of polyherbal formulation ACUPEN in experimental animal models. Methodology: The acute toxicity test of the Polyherbal formulation ACUPEN was performed by OECD 423-guideline. Analgesic activity was evaluated using Eddy's hot plate method & acetic acid-induced abdominal writhing test. The anti-inflammatory activity was measured using a digital vernier calliper with carrageenan-induced hind paw oedema. All drugs were given 1ml of 1% vehicle (CMC), Pentazocine 10mg/kg (i.p), Diclofenac 10mg/kg and Test drugs at doses 150, 300 and 600 mg/kg in 1% CMC (vehicle) were administered per orally in the experiment. Result and Discussion: Eddy's Hot plate method results showed central analgesic activity through a long-lasting significant increase in response time from 30 minutes to 120 minutes in the Polyherbal formulation ACUPEN. In acetic acid-induced writhing ACUPEN exhibited dose-dependent peripheral analgesic activity. Amongst low, mid and high, the high dose (600mh/kg) showed significant ($p<0.05$) peripheral analgesic activity compared to standard drug. In the carrageenan-induced paw oedema method, the percentage inhibition of all the groups was compared at 1, 2, 3, 4, and 5 h, and it was found that high-dose ACUPEN (600mg/kg) showed highly significant $P< 0.05$ of % inhibition than the other groups. Conclusion: From the current study it has been concluded that the Polyherbal formulation ACUPEN was found to be safe for greater than 5000mg/kg body weight. The formulation exhibited both central and peripheral analgesic action in a dose-dependent manner in experimental models. The Polyherbal formulation has also exhibited anti-inflammatory activity too.

Keywords: ACUPEN tablets, Analgesic, Anti-inflammatory, Polyherbal.

Introduction

Despite significant advances in medical science over the last few decades, many disorders, such as inflammatory disease, remain difficult to treat. Currently, conditions like Rheumatoid arthritis, Osteoarthritis, and neuropathic pain require long-term, safer and effective pain management. Due to the considerable adverse effects of steroidal and NSAID drugs, natural chemicals such as nutritional supplements and herbal therapies, which have been used for millennia to decrease pain and inflammation, are gaining in popularity. Natural anti-inflammatory substances have been used to mediate the inflammatory process for ages, and they have fewer negative effects than synthetic compounds. Herbal therapy outperformed synthetic medications in terms of safety, efficacy, cultural acceptance and side effects (1).

ACUPEN is the polyherbal formulation by ATBU HARITA Pharmaceutical Pvt. Ltd Vyara. The Formulation is designed to treat joint pain and inflammation associated with conditions like gout, rheumatoid arthritis, and osteoarthritis. The ingredients incorporated in this formulation already possess analgesic and anti-inflammatory activity (2-7). However establishing a polyherbal formulation requires preclinical safety and efficacy evaluation with suitable models. The formulation is intended to be useful in inflammatory disease conditions. Each tablet of ACUPEN contains the following ingredients in 300 mg oral tablet dosage form mentioned in Table 1. The formulation is required to be stored in a cool and dry place, away from direct sunlight.

*** Corresponding Author:**
Atul Desai
Ayurveda Physician,
Dhanvantari Clinic, Ayurveda Health Care and Research Centre, Vyara, Gujarat, India.
Email Id: dratuldesai@rediffmail.com

Materials and Methods

The study was approved by IAEC of ROFEL Shri G.M. Bilakhia College of Pharmacy Vapi with protocol number ROFEL/IAEC/2022/15. Wistar male rats weighing between 150-250 gm were used for the study. They were fed with a standard laboratory diet and

water ad libitum. Twelve hours of dark-light cycles were maintained.

Table 1: Polyherbal ACUPEN ingredients information

Sr. no	Constituents	Content	Parts Used
1	*Aloe vera* (L.) Burm.f.	50mg	Leaf
2	*Commiphora mukul* (Hook. ex Stocks) Engl.	40mg	Gum
3	*Apium leptophyllum* (Pers.) F.Muell.	30mg	Fruit
4	*Ricinus communis* L.	30mg	Root
5	*Myristica fragrans* Houtt.	20mg	Seed
6	*Boerhavia diffusa* L.	60mg	Root
7	Triphala	50mg	Powder
8	Excipients	20mg	-

Acute oral toxicity study

The acute oral toxicity study was determined using the OECD 423 toxic class method guideline. As per above mentioned guideline, three healthy female Wistar rats were orally administered ACUPEN employed 5000 mg/kg considering the available information on mortality is unlikely at the highest starting dose level. Animals were monitored for changes mentioned in OECD 423 guidelines (8).

Eddy's Hot Plate (9, 11)

Wistar rats were grouped as mentioned in Table 2. Rats will be placed on a hot plate with an electrically heated surface and kept at 55°±1°C. The Basal time was observed by placing the animal on a hot plate instrument and noting behaviours including jumping, withdrawal, and paw licking (any of the first reactions). The response time will be defined as the time when the animals lick their paws or leap, whichever came first, in response to the pain stimulus. A 15-second cut-off interval was implemented to avoid paw damage. The stopwatch will record the latency duration between when the animals are placed and when responses occur. The medications were given orally, and the latency period response was recorded at intervals of 0, 30, 60, 90, and 120 minutes, after which data were calculated or compared using the equation.

Table 2: Details on grouping and dosing of animals

Group no	Group name	Treatment	Dose	Route	No of animal
1	Control	Vehicle	1ml	p.o	6
2	Standard	Pentazocine	10mg/kg	i.p	6
3	Test 1	ACUPEN	150mg/kg	p.o	6
4	Test 2	ACUPEN	300mg/kg	p.o	6
5	Test 3	ACUPEN	600mg/kg	p.o	6
Where p.o.= per os (mouth/orally), i.p= intraperitoneal					

Evaluation:

$$\text{\% Inhibition of reaction time} = \frac{(\text{Mean reaction time of the test sample} - \text{Mean reaction time of the control group})}{\text{Mean reaction time of the control group.}}$$

Acetic Acid Induced Writhing Method (12-14)

Before the delivery of acetic acid intraperitonally, a group of animals (mentioned in Table 3) will be given the treatment formulations. Each animal will receive 10ml/kg of body weight of acetic acid (0.7 % v/v) administered intraperitonally. The animal replied by stretching, which is defined as a series of constrictions that migrate down the abdomen wall, turning motions, or hind leg extension. This behaviour will be monitored for 15 minutes and the response will be recorded. For 15 minutes, the total number of writhing will be recorded, and the period with the largest (%) of inhibition will be designated peak time.

Table 3:- Details on grouping and dosing of animals.

Group No	Group Name	Treatment	Dose	Route	No of Animal
1	Control	CMC	1ml	p.o	6
2	Standard	Diclofenac	10 mg/kg	p.o	6
3	Test 1	ACUPEN	150mg/kg	p.o	6
4	Test 2	ACUPEN	300mg/kg	p.o	6
5	Test 3	ACUPEN	600mg/kg	p.o	6

Evaluation:

$$\text{\% of inhibition} = \frac{\text{in control group} - \text{writhes in test group}}{\text{Writhes in the control groups}} \text{x100}$$

Carrageenan Induced Paw oedema (15-20):

By injection, the paw oedema was induced with 0.1 ml of 1% carrageenan in 0.9% saline in rats (mentioned in Table 4) at the sub-plantar region of the left hind paw. The treatment drugs were administered 30 min before carrageenan injection. The paw-induced thickness length will be measured by using a digital vernier calliper instrument. The measurement will be taken at different intervals of time from 0 hr (before carrageenan injection) and 1, 2,3,4,5 hr. later. The total inhibitions were calculated by using the equation.

Table 4: Details on grouping and dosing of animals

Group no	Group name	Treatment	Dose	Route	No of animal
1	Control	Cmc	1ml	p.o	6
2	Standard	Diclofenac	10mg/kg	p.o	6
3	Test 1	ACUPEN	Low	p.o	6
4	Test 2	ACUPEN	Mid	p.o	6
5	Test 3	ACUPEN	High	p.o	6

Evaluation:

% inhibition of paw oedema= (Vc-Vt\Vc) ×100

Where, Vc=average paw thickness of control group, Vt=average paw thickness of test group.

Statistical analysis

The results were expressed as the mean±SEM. The results obtained from the present study were analysed using one-way ANOVA followed by Bonferroni multiple comparison tests.

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Results and Discussion

Acute Toxicity study

Limit dosage studies up to 5000 mg/kg in rats were conducted for the polyherbal formulation ACUPEN, concerning paragraphs 22 and 23 of the OECD standards 423. The results showed that neither clinical changes nor death markers were seen. The observation includes no significant morbidity and mortality observed during the 14 days or gross abnormality and hence histopathology was not done.

Eddy's Hot Plate Method

The highest nociceptive inhibition was exhibited by ACUPEN (600 mg/kg) at 30 min presented in Table 5.

Table 5: Effect of treatment on reaction time in hot plate method

		Reaction time in seconds				
Group name	**Treatment**	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control	1ml of 1% cmc	3.00 ±0.39	3.30 ±0.34	3.10±0.62	3.03±0.43	3.03±0.43
Standard	Pentacozine(10mg/kg)	3.38±0.63*	13.20±0.30*	12.03±0.64*	10.03±0.80*	9.33±0.77*
Test-1	ACUPEN(150mg/kg)	3.30±0.38*	11.56±0.53*	11.45±0.41*	9.03±0.89*	8.52±0.44*
Test-2	ACUPEN(300mg/kg)	3.64±0.47*	12.04±0.59*	11.03±0.42*	9.03±0.66*	8.55±0.29*
Test-3	ACUPEN(600mg/kg)	3.84±0.84*	15.10±0.30*	14.99±0.40*	12.03±0.56*	11.20±0.47*

All data are expressed as mean ± SEM (n=6 in each group) and analysed by one- way ANOVA followed by Bonferroni multiple comparison test Where, C = Control, S= Standard (pentazocine 10mg/kg), T1= ACUPEN (150mg/kg) T2= ACUPEN (300mg/kg), T3= ACUPEN (600mg/kg). *P<0.05 when compared with control.

Eddy's hot plate result showed central analgesic activity through a sustained significant increase in response time from 30 to 120 minutes in ACUPEN as compared to control. The animals pretreated with ACUPEN showed a dose-dependent increase in latency of response in the hot-plate method. All treatment groups exhibit central analgesic activity for a longer period. The effect is equivalent to the standard drug Pentazocin. The possible reason behind the early onset of the analgesic effect is that ACUPEN consists of Triphala as one of the components of the formulation. Prior research has demonstrated that Triphala can produce an early-onset analgesic effect compared to standard medicine (21). This impact might help to overcome the drawback of polyherbal formulation in inducing delayed onset of response in some chronic and emergency inflammatory conditions.

Table 6: Effect of ACUPEN on acetic acid induced writhing method

Group	**Dose**	**No. of writhes**	**% Inhibition**
Control	1ml of 1% cmc	43.5 ±18.13	-
Standard	Diclofenac (10mg/kg)	4.5±1.35*	90.75
Test-1	ACUPEN (150mg/kg)	3.25±0.39*	94.22
Test-2	ACUPEN (300mg/kg)	3.0±0.74*	95.95
Test-3	ACUPEN (600mg/kg)	1.75±0.84*#	98.26

All data are expressed as mean ± SEM (n=6 in each group) and analyzed by one- way ANOVA followed by Bonferroni multiple comparison test.*P<0.05 when compared with control, # P<0.05 when compared with standard.

Acetic Acid Induced Writhing Models

Acetic acid-induced abdominal constriction is a sensitive technique and local peritoneal receptors are considered to be involved in this reaction which increases the content of PGE 2 and PGF 2 in the peritoneal fluid. Pain is produced indirectly in this technique by endogenous mediators such as prostaglandins, which excite peripheral nociceptive neurons. Both opioids and nonsteroidal anti-inflammatory medications affect these neural fibres. The release of many mediators, including bradykinin, substance-P, and PGs, as well as cytokines including IL1, TNF, nitric oxide, and IL8, is triggered by the local irritation generated by intraperitoneal delivery of acetic acid.

The oral administration of the ACUPEN drug showed a dose-dependent analgesic activity. The intraperitoneal injection of acetic acid caused a strong nociceptive response in the control group rats mentioned in Table 6. Amongst treatment groups, in all three doses, 600 mg/kg of ACUPEN exhibited statistically higher analgesic activity than the standard drug Diclofenac sodium 4.5±1.35 (90.75 % recovery). It was observed that the onset of writhing was delayed and the duration of writhing was shortened. Hence, ACUPEN 600mg/kg was found to be more significant when compared with standard and control.

Carrageenan induced paw oedema

In the carrageenan-induced model, the first phase of carrageenan-induced oedema is mediated by the release of histamine and serotonin, with a peak value of 1 hour; the second phase is mediated by the production of prostaglandins and leukotrienes, with a peak value of 3 hours. Treatment groups have suppressed the inflammation in both phases for longer periods. Since the formulation in our study exhibited a significant inhibitory effect on the rat paw oedema mentioned in Table 7, it probably exerts an inhibitory effect on some of the mediators of inflammation induced by the carrageenan stimuli (22).

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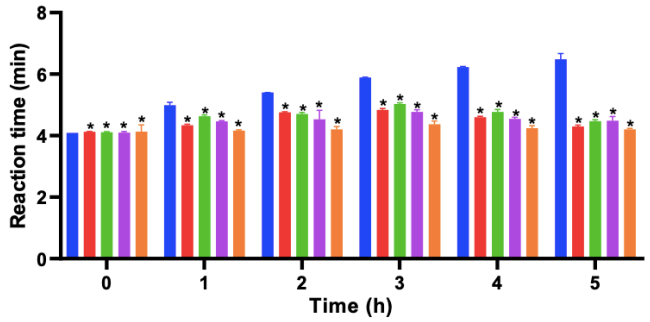
Table 7: Effect of ACUPEN in carrageenan induced paw oedema

Group Name	Paw thickness (mm)					
	0hr	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
Control	4.09 ±0.002	4.99±0.09	5.40±0.002	5.89±0.01	6.23±0.02	6.48±0.19
Standard	4.12±0.01*	4.34±0.02*	4.76±0.02*	4.84±0.04*	4.60±0.02*	4.30±0.04*
Test 1	4.11±0.02*	4.63±0.04*	4.70±0.05*	5.03±0.04*	4.77±0.07*	4.47±0.04*
Test 2	4.10±0.03*	4.46±0.03*	4.53±0.29*	4.77±0.07*	4.54±0.05*	4.48±14*
Test 3	4.13±0.22*#	4.16±0.02*#	4.20±0.09*#	4.37±0.10*#	4.24±0.08*#	4.20±0.03*#

All data are expressed as mean ± SEM (n=6 in each group). *P<0.05 when compared with control.
# P<0.05 when compared with standard

Figure 1: Reaction time in carrageenan induced paw oedema



[All data are expressed as mean ± SEM (n=6 in each group) and analyzed by one- way ANOVA followed by Bonferroni multiple comparison test. Where, ■ Control; ■Standard(10mg/kg);■Low dose(150mg/kg); ■Mid dose(300mg/kg); ■High dose(600mg/kg). *P<0.05 when compared with control]

Table 8: Effect of treatment on percentage of inhibition

Group Name	Paw thickness (% inhibition)					
	0 hr	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
Control (carrageenan)	0	0	0	0	0	0
Diclofenac 10mg/kg	0	13.02*	11.85*	17.82*	26.16*	33.64*
ACUPEN 150mg/kg	0	7.21*	12.96*	14.60*	23.43*	31.10*
ACUPEN 300mg/kg	0	10.26*	16.11*	19.01*	27.12*	30.86*
ACUPEN 600mg/kg	0	16.63* #	22.22* #	25.80* #	31.94* #	35.18* #

All data are expressed as mean ± SEM (n=6 in each group). *P<0.05 when compared with control., #P<0.05 when compared with standard

Figure 2: Paw with and without carrageenan

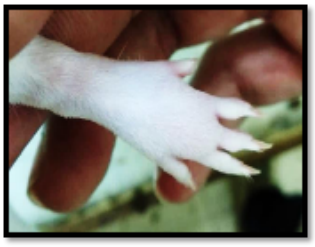
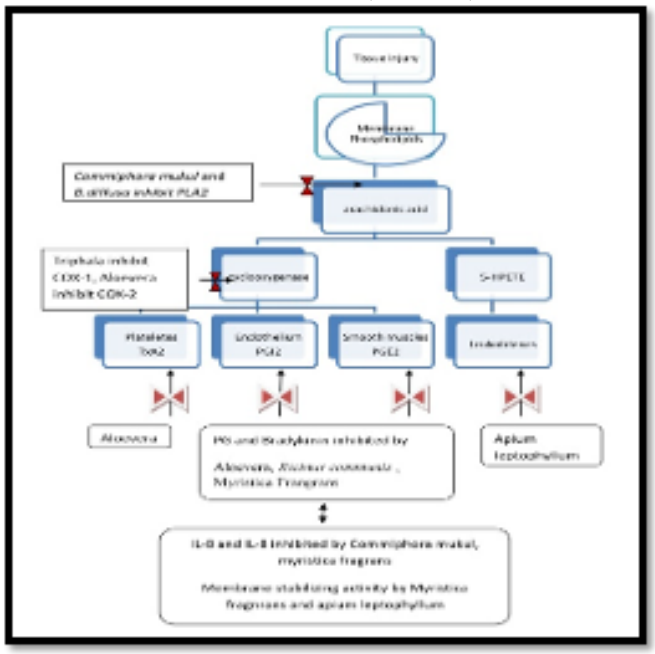
(a) Paw without injecting carrageenan	**(b) Paw after injecting carrageenan**
	

Figure 3: Possible role of ACUPEN in pain and inflammation (1, 23-24)



The individual herbs incorporated in the formulation exhibited a significant contribution to the synergetic mechanism as well as a faster onset of action and longer duration of action especially in Polyherbal formulations. Each ingredient of ACUPEN has their mechanism of action mentioned in Figure 3 for contributing as an effective analgesic and anti-inflammatory formulation. The activation and increased activity of the nitric oxide synthase (iNOS), COX-2 enzymes, and proinflammatory cytokines, such as tumour necrosis factor-alpha (TNF-), interleukin1 (IL-1), and interleukin (IL-6), among others, results in increased inflammatory mediator synthesis. To be considered anti-inflammatory, an agent must affect the outcomes of carrageenan-induced inflammation, resulting in the amelioration of symptoms such as oedema, pyrexia, redness, algesia, and tissue dysfunction. (26)

Conclusion

From the current study, it has been concluded that the Polyherbal formulation ACUPEN was found to be safe for greater than 5000mg/kg body weight. The formulation exhibited both central and peripheral analgesic action in experimental models. It has also shown anti-inflammatory activity. ACUPEN (600mg/kg) exhibits more significant peripheral analgesic

activity when compared with standard drug diclofenac. It is also important to make a note that the formulation exhibits analgesic and anti-inflammatory activity in a dependent manner. Key observations are faster onset and longer lasting analgesic and anti-inflammatory potential of ACUPEN deserve major attention. Some proposed mechanisms by which ACUPEN mediates its anti-inflammatory and analgesic benefits include change or antagonization in prostaglandin and leukotriene, membrane stability, and anti-oxidant action. Furthermore, pharmacological exploration is required to evaluate anti-rheumatic potential and inflammatory disorders.

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Conflict of Interest: Authors affirm no conflict of interest.

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