ejpmr, 2023,10(2), 228-233

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION FOR ANTI-PYRETIC, ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF SYNTHETIC PIPERONAL DERIVATIVES

A. Mamatha[1]*, K. Venkateshwarlu[2] and Ch. Suresh[3]

[1]Assistant Professor, Department of Pharmacology, Vijay College of Pharmacy, NH16 Borgaon, Nizamabad, Telangana 503003.

[2]HOD, Department of Pharmaceutical Analysis, Vaagdevi College of Pharmacy, Hanmakonda.

[3]Principal, Department of Pharmaceutical Chemistry, Vijay College of Pharmacy, NH16 Borgaon, Nizamabad, Telangana 503003.

***Corresponding Author: A. Mamatha**

Assistant Professor, Department of Pharmacology, Vijay College of Pharmacy, NH16 Borgaon, Nizamabad, Telangana 503003.

Article Received on 12/12/2022 **Article Revised on 02/01/2023** **Article Accepted on 22/01/2023**

ABSTRACT

Recent studies have revealed the key roles of pro-inflammatory cytokines, such as tumor necrosis factor-a (TNF-a), interleukin-1b (IL-1b), IL-6 and IL-8 in the pathogenesis of RA.[20] In the present study, we showed that petroleum ether, ethyl acetate and methanolic extract at a dose of 50 and 100 mg/kg body weight significantly inhibit the progression of the rheumatoid arthritis in treated animals. The effect of the extracts was dose dependent and for a long period compared to the standard. Earlier observations by Rekha et al (2010) supported the alterations in the metabolic activities of diseased rats.[21] Earlier findings suggest that absorption of 14C-glucose and 14C-leucine in rat's intestine was reduced in inflamed rats and it shows that the anti-inflammatory drugs have corrected the decreased absorption capacity of intestine during inflammation.[22,23] The increased body weight during the treatment with diclofenac and the extracts of Cassia uniflora leaves observed in this work may be due to the restoration of the absorption capacity of the intestine. **Objective:** To evaluate the anti-pyretic, anti-inflammatory and analgesic activity of synthetic Piperonal derivatives. **Method:** The Piperonal derivatives were administered orally to animals at 100mg/kg dose. Anti-inflammatory activity was studied in male Wistar rats using carrageenan induced paw edema method. Analgesic activity was evaluated in mice by using hot plate method. Antipyretic activity was evaluated by using brewer's yeast induced pyrexia method. **Results:** The study showed that the piperonal derivatives were having anti-inflammatory activity at the 100mg/kg test doses. The analgesic activity was also found to significant ($p<0.05$) and dose dependent prolongation of response latency in the hot plate test. The antipyretic assay revealed the temperature decreasing pattern significantly ($p<0.01$) when compared with control. **Conclusion:** These results demonstrated that Piperonal derivatives have potential health benefits as it can produce significant anti-inflammatory, analgesic and anti-pyretic activity.

1. INTRODUCTION

In recent years there has been a large investigation on development of different Piperonal derivatives, many of which were found to possess an extensive spectrum of pharmacological activities. Piperonal is a chemically 3, 4-(methylenedioxy) benzaldehyde or 1, 3-benzaldehyde-5-carboxaldehyde. It contains a methylenedioxy group and an aldehyde group. It can be prepared by oxidizing piperic acid with potassium permanganate; by condensing methylene iodide with protocatechuic aldehyde or by oxiding isosfrole with chromic acid. Piperonal occurs as long white transparent crystals. Melting point is 37^0C. Boiling Point is 263^0C and it is slightly soluble in cold water but readily soluble in alcohol and ether. The derivatives are Test compound 1:4-((6-(benzo[d][1,3]dioxol-5-yl)-4-phenyl-1,4-dihydropyrimidine-2-yloxy) methyl) morpholine, Test compound 2:4-((6-(benzo[d][1,3]dioxol-5-yl)-4-p-tolyl-1,4-dihydropyrimidine-2-yloxy) methyl) morpholine and Test compound 3: 4-((6-(benzo[d][1,3]dioxol-5-yl)-2-(morpholinomethoxy)-1,4-dihydropyrimidine-4-yl)benzenamine. Piperonal derivatives have been reported for treatment of cancer, antibacterial, antifungal, antiviral, anti-convulasant, anti-malarial, anti-hypertensive, anti-inflammatory, antipyretic, antidepressant, analgesic agent. As part of our ongoing program in the development of novel lead molecules with antinflammatory, analgesic and antipyeretic activity we evaluated the piperonal derivatives.

2. MATERIALS AND METHODS

2.1. Drugs and Chemicals: The following drugs namely, Paracetamol, Diclofenac and carrageenan were used during the experimental study.

2.2. Experimental animals: Male albino wister rats (150-200g) and swiss albino mice (20-30g) were used for the experimental study. The animals were maintained under standard husbandry conditions in

polypropylene cages and provided with food and water *ad libitum*. The animals were kept on fasting overnight prior to the experimentation and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

2.3. Acute toxicity studies: The acute toxicity was performed according to OECD guideline (423). The male albino mice (20-25g) were selected to find out the acute toxicity study of Piperonal derivatives. Each animal received single dose of test compound (300 mg/kg, 5% gum acacia) based on the classic (OECD Guidelines-423) method of CPCSEA. Animals were observed individually and continuously for 30min, 2hr and 24hr to detect changes in the autonomic or behavioral responses and also for tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and monitored mortality for the following 14 days.

2.4. Antipyretic activity

Yeast induced pyrexia in rats

The antipyretic activity of Test compound 1, Test compound 2 and Test compound 3 were evaluated by using telethermometer. Male wistar rats (150-200gm) were selected and divided into five groups each having five animals. They were maintained at standard constant temperature of 97-98^{o} F for 24 hours before pyrexia was induced. Experimental pyrexia induced with 15% suspension of Brewer's yeast in 2% gum acacia in normal saline was given 0.25ml/100gm dose. The rectal temperature before and after treatment was recorded with the help of digital clinical thermometer at every hour up to three hours was compared with control. Before yeast injection the body temperature was recorded. After 18hr of yeast injection, control and the test drugs at a dose 100mg/kg were administered orally to each group as a suspension in saline, standard drug Paracetamol (10mg/kg) was also administered to a group. Rectal temperature was noted using telethermometer. The probe was inserted 3-4cm deep into the rectum, by lubricating the tip of telethermometer with a lubricant (wax). Inserted the lubricated telethermometer into the anal opening ½ inch to 1 inch after fastened the tail and recorded the rectal temperature.

2.5. Anti-inflammatory activity

Carrageenan induced paw edema

Male albino wistar rats (150-200g) were divided into five groups, each group contain five animals. Edema was induced by injecting carrageenan (1% w/v, 0.1ml/paw) in the right hind paw of rats. The Test drugs i.e. Test compound 1(100mg/kg), Test compound 2(100mg/kg), Test compound 3(100mg/kg), Diclofenac (10mg/kg), or vehicle was administered orally 1h before injection of carrageenan. Paw volume was measured with digital Plethysmometer (Model No. 7140, Ugo Basile Srl, Comerio, Italy) after 1st, 3rd and 4th hr injection.

The % increase in paw volume was calculated by the formula,

Percentage increase = A-B/A×100

A: Paw volume at different time points after injection

B: Paw volume before injection

2.6. Analgesic activity

Eddy's Hot plate method:

The albino mice were divided into five groups, each group contains five animals. Three groups received the Test compound 1 (100mg/kg), Test compound 2 (100mg/kg) and Test compound 3(100mg/kg), while the remaining two groups received saline (control) and Diclofenac (10mg/kg). The Test drugs, saline or Diclofenac were administered orally to the animals after 12h of fasting. The animals were each placed on a hot plate maintained at 55 ±0.5^{0}C, 30min after administration of Test compounds, saline or Diclofenac. The time taken for the rats to respond to the thermal stimulus (usually by jumping) was noted as the latency (in seconds). The mean of the latency for each group was determined. The effects of the test drugs, Diclofenac and saline were also determined after 30, 60 and 90min of administration to rats. Cut off time was 20 seconds.

2.7. Statistical analysis

Results are expressed as mean SEM. Statistical significance was determined by using the one way ANOVA followed by Dunnett's multiple comparison test. P< 0.05 was considered statistically significant.

3. RESULTS

3.1. Acute toxicity

The oral median lethal dose (LD50) of the test compound 1, test compound 2 and test compound 3 were found to be 500mg/kg, 1000mg/kg and 1000mg/kg body weight in mice.

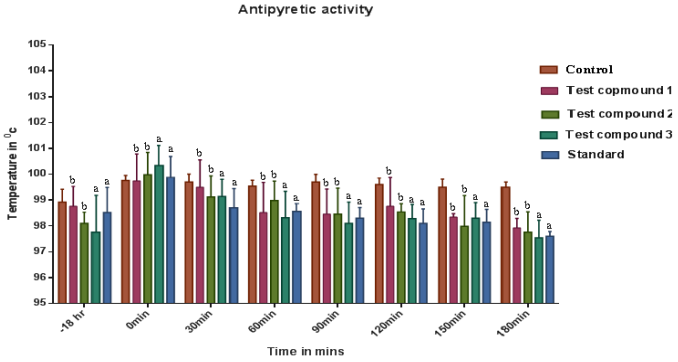
3.2. Yeast induced pyrexia in rats

Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration. Treatment with the Test compound 1(100mg/kg), Test compound 2(100mg/kg) and Test.

Compound 3(100mg/kg) significantly decreased the rectal temperature of the rats. The antipyretic effect started as from the first hour and the effect was maintained for 4h, after administration of the test compounds. The result obtained from both the standard (Paracetamol) and Test compounds were compared with that of control and a significant reduction in the yeast induced elevated rectal temperature was observed.

Table 1: Rectal temperature (^{0}F) of different treated groups.

Group and Dose (n=5)		Rectal temperature in ˚F at different time points (MEAN±SD)						
	-18[a]hr	0min	30min	60min	90min	120min	160min	180min
Control	98.92± 0.49	99.76± 0.19	99.70± 0.30	99.54± 0.23	99.70± 0.29	99.60± 0.25	99.50± 0.30	99.50± 0.20
Standard (paracetamol)	98.52± 0.97	99.88± 0.80	98.70± 0.75	98.56± 0.30[a]	98.30± 0.40[a]	98.10± 0.56[a]	98.14± 0.49[a]	97.60± 0.19[a]
Testcompound1 100 mg/kg	98.762± 0.76	99.740± 1.04	99.50± 1.05	98.52± 1.16[b]	98.46± 0.96[c]	98.76± 1.11[b]	98.34± 0.13[b]	97.92± 0.37[b]
Testcompound2 100 mg/kg	98.10± 0.43	99.98± 0.86	99.12± 0.81	98.98± 0.75[b]	98.46± 1.00[b]	98.54± 0.32[b]	97.98± 1.19[c]	97.76± 0.78[b]
Testcompound3 100 mg/kg	97.76± 1.42	100.34± 0.77	99.14± 0.66	98.32± 1.01[a]	98.10± 0.82[b]	98.28± 0.55[a]	98.30± 0.60[a]	97.54± 0.68[a]

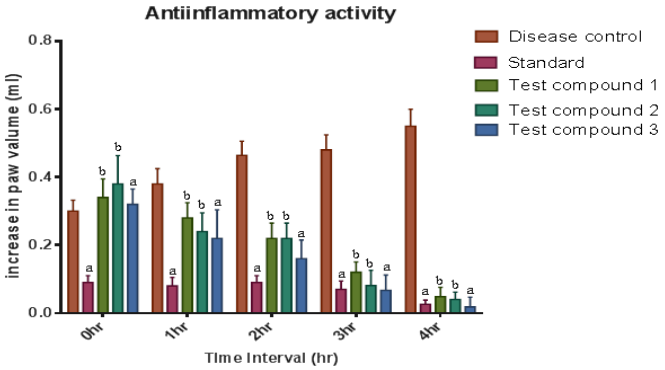


3.3. Carrageenan induced paw edema

Pretreatment with Test compound 1, Test compound 2 and Test compound 3 significantly prevented increase in volume of paw edema at 100mg/kg dose. The Test compound 3 was able to effectively inhibit increase in paw volume than Test compound 1 and Test compound 2 when compared with control.

Table 2: Increase in paw volume with time.

Group and dose	Increase in paw volume (ml) at different time intervals				
	0.5hr	1hr	2hr	3hr	4hr
Control	0.3±0 032	0.38±0. 044	0.46±0. 041	0.48±0. 044	0.55±0. 050
Standard(Diclofenac) 10mg/kg	0.09±0. 020***	0.08±0. 024***	0.090±0. 020***	0.070±0. 024***	0.026±0. 012***
Test compound1 100mg/kg	0.34±0. 054*	0.28±0. 044**	0.22±0. 044**	0.12±0. 030*	0.048±0. 0273**
Test compound2 100mg/g	0.38±0. 083**	0.24±0. 055**	0.22±0. 447**	0.081±0. 044**	0.039±0. 022**
Test compound3 100mg/kg	0.32±0. 044***	0.22±0. 084***	0.16±0. 054***	0.067±0. 044***	0.018±0. 0288***

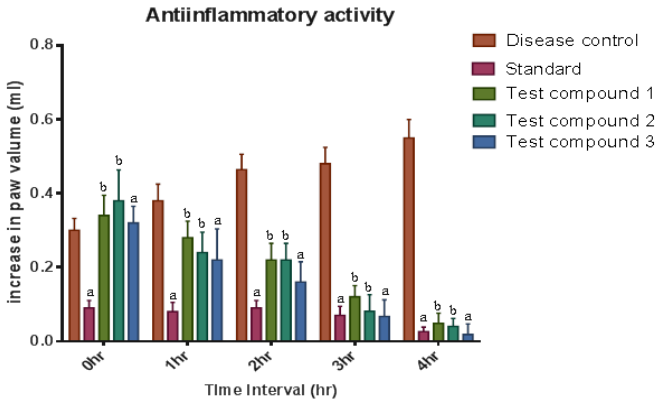


3.4. Hot plate latency method

The effect of Test compound 1, Test compound 2 and Test compound 3 at 100mg/kg were evaluated for analgesic activity. Table shows results of thermal stimulus induced pain (Eddy's hot plate) in mice. Pretreatment with diclofenac or test drugs did not produce any significant changes in paw licking time in the early phase of pain. However, in the late phase, significant ($P<0.01$) increase in licking time was observed in mice treated with test compounds and standard. The maximum activity was observed with Test compounds (100mg/kg) at the 60min time interval when compared to the standard diclofenac.

Table 3: Reaction time (sec) of different treated groups.

Group (n=6)	Reaction time in min at different time points(mins) (mean±SD)				
	Treatment	**0**	**30**	**60**	**90**
I	Control	5.20±2.129	6.27±0.165	6.00±0.374	5.27±0.487
II	Test compound 1 100mg/kg	7.05±0.443	7.10±0.365*	7.20±0.346*	7.14±0.431*
III	Test compound 2 100mg/kg	7.55±0.854	7.98±1.275*	8.26±1.697**	8.56±2.003**
IV	Test compound 3 100mg/kg	6.36±1.312	6.45±1.056**	6.55±1.121**	7.10±1.823**
VI	Diclofenac10mg/kg	10.55±0.998	11.50±1.329***	13.22±1.114***	14.34±0.695***



4. DISCUSSION

The hot-plate test is useful in elucidating centrally mediated anti-nociceptive responses, which focuses mainly on changes above the spinal cord level. The significant increase in pain threshold produced by petroleum ether extract of *Cassia uniflora* suggests involvement of central pain pathways. Pain is centrally modulated *via* a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems. The analgesic effect produced by the extract may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in inflammation and pain.[10,11]

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. The method has also been associated with prostanoids in general, that is, increased levels of prostaglandin-E2 (PGE2) and PGF2秭 in peritoneal fluids as well as lipoxygenase products.[12] The significant reduction in acetic acid-induced writhes by petroleum ether extract, ethyl acetate extract and methanolic extract of Cassia uniflora Mill. suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 to 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase (3 h) is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymerphonu clear cells and prostaglandins produced by tissue macrophages.[13,14] The significant ($P<0.05$) suppressive activity of the different extracts of Cassia uniflora leaves in late phase shows its potent anti-inflammatory effect. This result is quite similar to the one observed for diclofenac at 20 mg/kg, which inhibited the edema by 68.19%. The results were statistically significant ($P<0.05$). Ueno et al (2000) found that the injection of

carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandin and other autacoids, which are responsible for the formation of the inflammatory exudates.[15] Besides, in the carrageenan-induced rat paw oedema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism.[16] PGE2, a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to the redness and increased blood flow in areas of acute inflammation. Therefore, it is suggested that the mechanism of action of the extracts may be related to histamine and prostaglandin synthesis inhibition.

The results of the present study also indicate that different extracts of Cassia uniflora leaves exhibits anti-arthritic effects in rats with Freund's adjuvant-induced arthritis. The model of adjuvant induced arthritis in rats has been extensively used in the study of inflammatory processes.[17] Freund adjuvant is an antigen solution emulsified in mineral oil that is used as an immune-potentiator. The complete form (CFA) is composed of inactivated and dried mycobacteria and is effective in stimulating cell mediated immunity and may lead to the potentiation of the production of certain immunoglobulins. Shortly after the administration of CFA into hind paw, pronounced swelling appears in the hind paw which persists for weeks (primary reaction). After few days, the contralateral paw as well as front paw also becomes swollen and arthritic nodules appear in ear and tail (delayed systemic response).[18,19] Rheumatoid arthritis (RA), which is associated with systemic inflammatory disorders, is a chronic inflammatory disease involving multiple joints. It is an autoimmune disorder of unknown etiology that is characterized by progressive joint destruction, deformity, disability and premature death in most patients. Recent studies have revealed the key roles of pro-inflammatory cytokines, such as tumor necrosis factor-a (TNF-a), interleukin-1b (IL-1b), IL-6 and IL-8 in the pathogenesis of RA.[20]

In the present study, we showed that petroleum ether, ethyl acetate and methanolic extract at a dose of 50 and 100 mg/kg body weight significantly inhibit the progression of the rheumatoid arthritis in treated animals. The effect of the extracts was dose dependent and for a long period compared to the standard.

Earlier observations by Rekha et al (2010) supported the alterations in the metabolic activities of diseased rats.[21] Earlier findings suggest that absorption of 14C-glucose and 14C-leucine in rat's intestine was reduced in inflamed rats and it shows that the anti-inflammatory drugs have corrected the decreased absorption capacity of intestine during inflammation.[22,23] The increased body weight during the treatment with diclofenac and the extracts of Cassia uniflora leaves observed in this work may be due to the restoration of the absorption capacity of the intestine.

The potent anti-arthritic effect of extracts is further Sheetal S. Chaudhari et al ./Asian Pacific Journal of Tropical Biomedicine (2012)S181-S186 S186 confirmed by radiological studies. The diagnosis of RA is usually obvious clinically and it allows therapeutic monitoring which remains the standard method in evaluating disease progression. The X-ray appearance, commonly referred to as to as diminished joint space is the hallmark as diminished joint space is the hallmark of arthritis.[24] In control rats, erosion representing bony destruction were evident on bone unprotected by cartilage, since they are exposed directly to cytokines such as TNF-毬 and IL-1 which stimulate the chrondocytes to produce proteolytic enzymes such as collagenases, glycohydrolases and neutral proteases degrading the cartilage. As a result, the pannus invades the joint and sub-chondral bones and eventually the joint is destroyed and undergoes fibrous fusion or ankylosis. These changes were reverted back to near normal upon petroleum ether and methanol extracts treatment.

5. CONCLUSION

These results demonstrated that Piperonal derivatives have potential health benefits as it can produce significant anti-inflammatory, analgesic and anti-pyretic activity.

REFERENCES

1. Nomenclature of Organic Chemistry: IUPAC Recommendations and Preferred Names 2013 (Blue Book). Cambridge: The Royal Society of Chemistry, 2014; 392. doi: 10.1039/9781849733069-FP001. ISBN 978-0-85404-182-4.
2. Jakubke, Hans Dieter; Jeschkeit, Hans Concise Encyclopedia of Chemistry. Walter de Gruyter, 1994; 1–1201. ISBN 0-89925-457-8.
3. DHHS (NIOSH) Publication No, 2019; 2: 2016–171.
4. Jump up to:[a] [b] Hoydonckx, H. E.; Van Rhijn, W. M.; Van Rhijn, W.; De Vos, D. E.; Jacobs, P. A. "Furfural and Derivatives". Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH. doi: 10.1002/14356007.a12_119.pub2.
5. Senning, Alexander Elsevier's Dictionary of Chemoetymology. Elsevier, 2006. ISBN 0-444-52239-5.
6. Limpricht, H. "Ueber das Tetraphenol C_4H_4O". Berichte der Deutschen Chemischen Gesellschaft, 1870; 3 (1): 90–91. doi:10.1002/cber.18700030129.
7. Rodd, Ernest Harry Chemistry of Carbon Compounds: A Modern Comprehensive Treatise. Elsevier, 1971.
8. Wilson, W. C. "Furan". Organic Syntheses.; Collective, 1941; 1: 274.
9. Hou, X. L.; Cheung, H. Y.; Hon, T. Y.; Kwan, P. L.; Lo, T. H.; Tong, S. Y.; Wong, H. N. "Regioselective

syntheses of substituted furans". Tetrahedron, 1998; 54 (10): 1955–2020. doi: 10.1016/S0040-4020(97)10303-9.

10. Katritzky, Alan R. (2003). "Synthesis of 2, 4-disubstituted furans and 4, 6-diaryl-substituted 2, 3-benzo-1, 3a, 6a-triazapentalenes" (PDF). Arkivoc, 2004; (2): 109–121. doi: 10.3998/ark.5550190.0005.208.