Original Research Article

Synthesis and evaluation of Anti-inflammatory activity of some Chalcone hydrazide derivatives

ABSTRACT

Aim: Chalcones are part of the selected group of chemical compounds related with diverse pharmacological activities such as antimicrobial, antiviral, antitumor, convulsion, anxiety, anti-inflammatory, andanalgesic. The aim of this study was to synthesize chalcones hydrazide derivatives and to evaluate their anti-inflammatory activity in the model of carrageenan induced edema in rats.

Methods: The chalcone hydrazide derivatives were prepared by treating Isonicotinyl and Nicotinyl hydrazide with respective parent chalconeand environmentally friendly solvents with 78–85% yields in approximately 6hrs. These compounds were characterized by 1H NMR, 13C NMR and IR spectroscopy. The structures of synthesised derivatives were evaluated on biological activity at dose of 40 mg/kg and they showed anti-inflammatory protective effect by oral administration, this effect was time dependent.

Results: All the derivatives were given satisfactory reaction yields that representing the efficiency of the employed synthetic route. In carrageenan-induced paw edema model, CL-2derivative showed most significant percentage of inhibition (61.97 %)as compared to reference standard Indomethacin.

Conclusion: Compound CL-2 was showed significant anti-inflammatory activity due to presence of electron donating groups like $-CH_3$on ring A and electron withdrawing group like NO_2 on ring B at 4^{th} position in isonicotinyl hydrazide derivative.All synthetic chalcone hydrazide derivatives may be considered as safer drugs for treating inflammatory conditions.

Keywords:*Chalcone, Inflammation, Hydrazide, Carrageenan,edema*

1. INTRODUCTION

Inflammation is an evolutionarily well preserved process characterized by the activation of immune and non-immune cells that protect the host from bacteria, viruses, toxins and infections by removing pathogens and promoting tissue repair and recovery.There are normally two types of inflammation: acute

and chronic inflammation. Inflammatory diseases include a huge array of conditions that are primarily characterized by inflammation. Common examples include allergy, inflammatory bowel disease, coeliac disease, asthma, autoimmune diseases, glomerulonephritis, perfusion injury, hepatitis, and transplant rejection etc. In the world, the most significant cause of death is chronic inflammatory disease [1-3]. The World Health Organization (WHO) ranks chronic diseases as the greatest threat to human health and further anticipated to increase persistently [4, 5].

Steroidal and Non-steroidal anti-inflammatory drugs known as NSAIDs are generally used in the management of acute and chronic pain of several etiologies, including cancer associated pain as well as arthritis. In the treatment of mild to moderate pain these drugs are used individually and in case of severe pain are used along with opioid analgesics or adjuvant analgesic drugs. For decades, physicians trusted on steroids to suppress immune response. Steroids are important anti-inflammatory agents, but reported to have severe adverse effects like enlargement of the heart, liver cancer, weight gain, etc. NSAIDs are non-selective cyclooxygenase enzymes (isoenzymes 1 and 2) inhibitors; pose a potentially serious risk with its acute and chronic use like gastrointestinal ulceration, hematologic toxicity, nephrotoxicity etc. The severity of NSAIDs adverse effect is experiencing more in the immuno-compromised patients suffering from life-threatening illness like cancer, HIV/AIDS etc. This is due to the occurrence of gastrointestinal bleeding and the masking of opportunistic infections related to the antipyretic effects of NSAIDs pose particular risk and might even cause fataldifficulties in patients who are thrombocytopenic,neutropenic, or else immuno-compromised. The ability of researchers to better understand the underlying cause of disease, identify group of patients, through accurate medicine, who will respond better to certain treatments, can potentially lead to new and innovative medicines[6].

Chalcones are present in high concentration in edible plants and precursors of flavonoids as well as isoflavonoids. Chalcones are intermediates in the aurones synthesis of flavones.Chemically they are open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system (1,3-diphenyl-2-propen-1-one). In the 21st century, scientist remains interested in chemistry of chalcone due to the presence of replaceable hydrogens that permits synthesis of large number of analogues and a variety of promising pharmacological activities to be generated such as anti-

oxidant[7],antimicrobial[8],antiprotozoal[9], anti-leishmanial[10,11], antimalarial[12,13],anti-HIV[14],anti-inflammatory[15,16],anticancer[17-19],anti-osteogenic[20]etc.

Current pharmacotherapy for inflammatory diseases reported limited therapeutic outcomes and are associated with many deleterious side effects. This indicates the need to develop new drugs for the treatment of inflammatory diseases with improved therapeutic outcome. Although the exact mechanisms of action of chalcone hydrazide analogues remain unknown, a study in inflammation indicated that chalcone can inhibit the action of COX-2. On this background, the main objective of the present study was to synthesize novel chalcone hydrazide derivativesand evaluated theiranti-inflammatory activityin the carrageenan induced paw edema model in rat.

2. MATERIAL AND METHODS

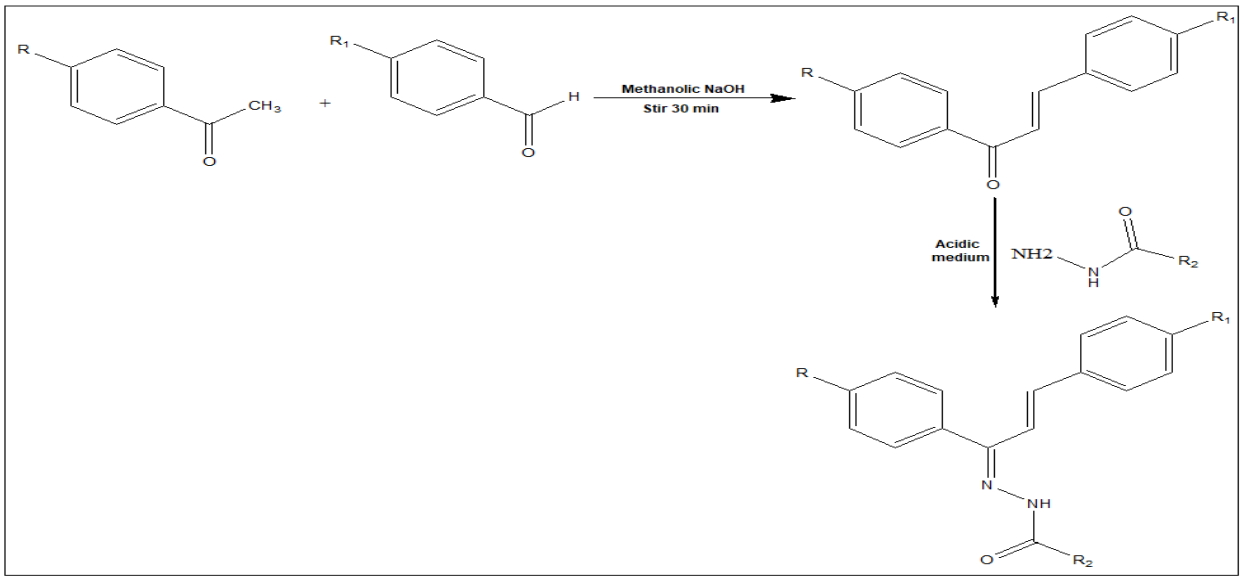
2.1 Material:

The chemicals employed in the synthetic work i.e. 4-NO_2- benzaldehyde, 4-Cl benzaldehyde, 4-4-isopropyl benzaldehyde, 4-Cl acetophenone, 4-Methyl acetophenone, Nicotinyl hydrazide, Isonicotinyl hydrazide, NaOH, Methanol, Conc. Acetic acid were purchased from Loba Chemicals Pvt Ltd, Mumbai.

2.2 Animals:

Male Wistar rats (200-250 gm) was procured from Lacsmi Biopharms Pvt. Ltd and housed under standard laboratory condition of 12:12 h light/dark cycle in a temperaturecontrolled (22 ± 2ºC) environment with ad libitum access to rodent chow and water. All experimental protocols were approved by Institutional Animal Ethics Committee (IAEC), Committee Constituted for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) by Ministry of Environment and Forests, Government of India, New Delhi, India (IAEC approval No. DYPCOP/IAEC/2021/8)

2.3 Synthesis:



Scheme 1: Synthetic scheme for chalcone hydrazide derivatives

Step 1: A Methanolic solution of substituted benzophenone and substituted Benzaldehyde were prepared, then mix both the solution and stir on magnetic stirrer. In this solution add 5-6 drops of saturated solution of NaOH and continue the stirring for 30 min. A parent Chalcones were obtained by this procedure.Completion of reaction was checked by thin layer chromatography using mobile phase Chloroform: Methanol (70:30).

Step 2: A Methanolic solution of chalcone was prepared then add Methanolic solution of appropriate hydrazide such as Nicotinyl hydrazide or Isonicotinyl hydrazide, maintained the acidic medium using acetic acid and solution was refluxed for about 6hrs. Completion of reaction was checked by thin layer chromatography using mobile phase Chloroform: Methanol (90:10).

2.4Physicochemical studies and characterization of synthesized compounds

Completion of reaction was checked by using thin- layer chromatographyusing Merck silica gel 60 F-254 plates had layer thickness about 0.25 mm and detection was performed in UV lamp and all solvents were distilled before use. All of the derivatives were characterized by IR, 1H, and 13C NMR spectra recorded with Bruker WM- 300 in deuterated DMSO at 400 and 100 MHz, respectively using tetra methyl silane (TMS) as the internal standard. All chemical shifts are described on the δ scale.

2.5 Anti-inflammatory activity:

Anti-inflammatory potency was analyzed using carrageenan-induced paw edema in rats. The rats were divided into 09 groups consisting six in each group. The first control group was given vehicle DMSO per oral 1ml/100gm body weight whereas third group served as reference standard received Indomethacin 20 mg/kg orally. The test compounds Chalcone derivatives were administered to groups 4^{th} to 9^{th} at the dose of 40 mg/kg per oral. The vehicle used for the preparation of the test compound was DMSO. After thirty minutes of above treatment, carrageenan solution 0.1 ml (1% w/v carrageenan dissolved in normal saline) injected into sub-plantar region of rat's left hind paw to induce inflammation. The group 2^{nd} received only carrageenan injection and served as induction control (Negative control) group.

Digital Plethysmometer was used to record the paw volume of control, reference standard and test compound treated groups and degree of paw edema measured at the interval of 1, 2, 3, 4 hours after carrageenan injection [21]. The percentage inhibition of edema was calculated using following formula.

$$\%\ \text{inhibition of edema} = \frac{[(\text{VT} - \text{V0})\text{control} - (\text{VT} - \text{V0})\text{Treatment}]}{(\text{VT} - \text{V0})\text{control}} \text{X}100$$

Where, V0: is paw volume at 0 hours and VT: is paw volume of respective time interval

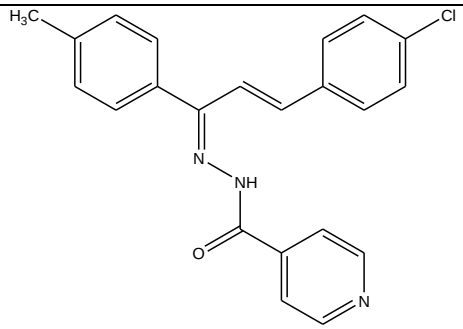
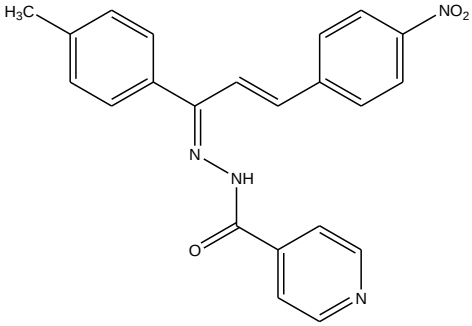
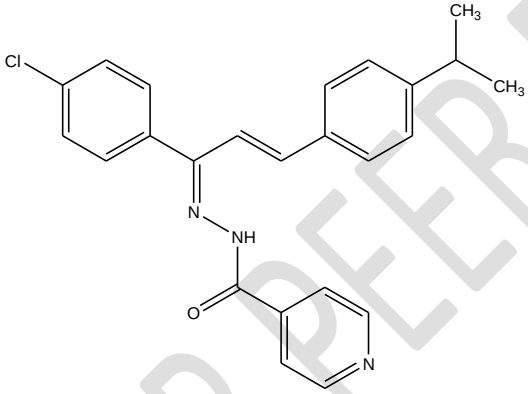
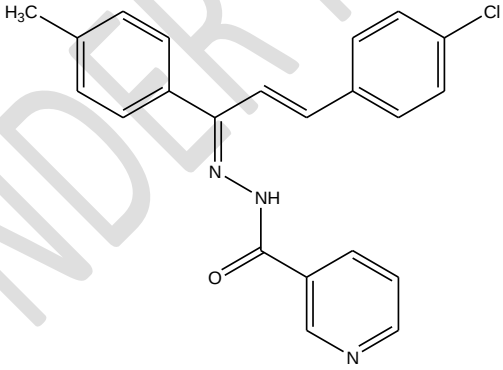
3. RESULTS AND DISCUSSION

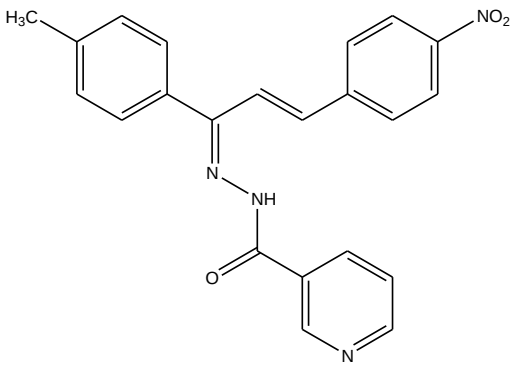
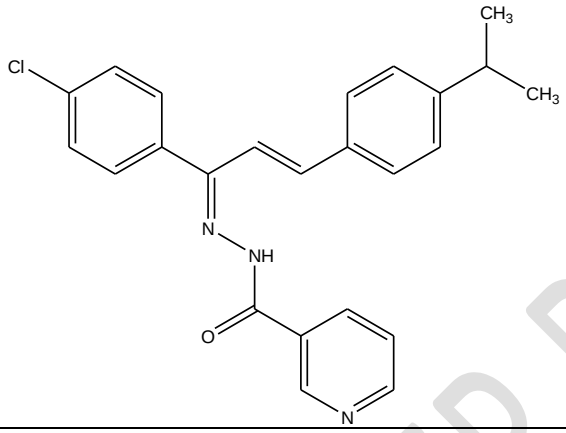
3.1 Physicochemical Characterization of Chalcone hydrazide Derivatives

The table below shows the physicochemicalcharacterization of the derivativesexaminedinthis study (Table 1). All the derivatives were properly separated from the reaction mixture and given satisfactory reaction yields, that representing the efficiency of the employed synthetic route.

Table 1: Physicochemical characteristics of Chalcone hydrazide derivatives

Compound	Structure	Yield (%)	Rf*	Melting Point (^{o}C)

CL-1		78	0.69	169-171
CL-2		82	0.75	155-157
CL-3		81	0.62	162-164
CL-4		82	0.72	150-152

CL-5		85	0.78	147-149
CL-6		84	0.80	161-163

Rf* Solvent system used for TLC was Chloroform: Methanol (90:10)

The Characterization of synthesized derivatives was carried out by using Infrared spectroscopy (IR), Proton NMR (H1 NMR) and Carbon NMR (C13 NMR) for structure elucidation.

CL-1:IR (KBr, cm^{-1}):3265(N-H), 1578 (C=N), 1524 (C=C aromatic), 1509 (C=C alkenyl), 745 (C-Cl).

NMR 1H 400 MHz (DMSO-*d6*):δ 2.20,3H (s), 6.63,1H (d, *J* = 17.9 Hz), 6.97-7.24,3H (7.08 (d, *J* = 17.9 Hz), 7.17 (ddd, *J* = 0.5, 1.3, 8.0 Hz)), 7.34,2H(ddd, *J* = 0.6, 1.5, 8.0Hz), 7.41-7.56,4H (7.49 (ddd, *J* = 0.6, 1.3, 8.0 Hz), 7.52 (ddd, *J* = 0.5, 1.8, 8.0Hz)), 7.93,2H(ddd, *J* = 0.4, 2.7, 4.5Hz), 8.71,2H(ddd, *J* = 0.4, 1.9, 4.5Hz).

NMR 13C (100 MHz, DMSO*d6*):127.8, 128.8, 129.1, 129.2 (8C; Benzene CH) 130.2, 133.3, 133.5, 140.7 (4C; Benzene-C), 155.6 (1C; Imine), 118.7, 139.0(2C; ethylene CH), 24.3 (1C; Aliphatic CH_3), 163(1C; amide), 122.8, 149.8 (4C; 4-Pyridine CH), 140.9 (1C; 4-Pyridine C).

CL-2: IR (KBr, cm^{-1}):3260(N-H), 1570 (C=N), 1522 (C=C aromatic), 1512 (C=C alkenyl), 342 (N=O).

NMR 1H 400 MHz (DMSO-*d*6):δ 2.22,3H(s), 6.89,1H (d, *J* = 17.5 Hz), 7.18-7.36,3H (7.25 (ddd, *J* = 0.5, 1.3, 8.0Hz), 7.29 (d, *J* = 17.5 Hz)), 7.54'2H,(ddd, *J* = 0.5, 1.7, 8.0Hz), 7.71, 2H (ddd, *J* = 0.5, 1.9, 8.7 Hz), 7.91, 2H (ddd, *J* = 0.4, 2.6, 4.5Hz), 8.28, 2H (ddd, *J* = 0.5, 1.8, 8.7Hz), 8.73, 2H (ddd, *J* = 0.4, 1.9, 4.5Hz).

NMR 13C (100 MHz, DMSO*d6*):121.0, 127.3, 129.1, 129.2 (8C; Benzene CH) 130.2, 140.7, 141.3, 147.6 (4C; Benzene-C), 155.6 (1C; Imine), 118.7, 139.0(2C; ethylene CH), 24.3 (1C; Aliphatic CH_3), 163(1C; amide), 122.8, 149.8 (4C; 4-Pyridine CH), 140.9 (1C; 4-Pyridine C).

CL-3: IR (KBr, cm^{-1}):3258(N-H), 1575 (C=N), 1525 (C=C aromatic), 3082 (Csp2 -H), 2938 (Csp3 –H), 1510 (C=C alkenyl), 740 (C-Cl).

NMR 1H 400 MHz (DMSO-*d*6):δ 1.16,6H (d, *J* = 7.0 Hz), 2.86, 1H (sept, *J* = 7.0 Hz), 6.65,1H (d, *J* = 17.9 Hz), 7.06,1H (d, *J* = 17.9 Hz), 7.26, 2H (ddd, *J* = 0.5, 1.6, 8.0Hz), 7.32-7.49, 4H (7.41 (ddd, *J* = 0.5, 1.8, 8.6Hz), 7.45 (ddd, *J* = 0.5, 1.9, 8.0Hz)), 7.61, 2H (ddd, *J* = 0.5, 1.5, 8.6Hz), 7.93,2H (ddd, *J* = 0.4, 2.7, 4.5Hz), 8.72, 2H (ddd, *J* = 0.4, 1.9, 4.5Hz).

NMR 13C (100 MHz, DMSO*d6*): 126.1, 129.0, 130.6 (8C; Benzene CH) 131.3, 132.4, 136.6, 147.8 (4C; Benzene-C), 155.6 (1C; Imine), 118.7, 139.0(2C; ethylene CH), 23.4 (2C; Aliphatic CH_3), 36.3 (1C; Aliphatic CH), 163(1C; amide), 122.8, 149.8 (4C; 4-Pyridine CH), 140.9 (1C; 4-Pyridine C).

CL-4:IR (KBr, cm^{-1}): 3260(N-H), 1569 (C=N), 1525 (C=C aromatic), 1512 (C=C alkenyl), 749 (C-Cl).

NMR 1H 400 MHz (DMSO-*d*6):δ 2.21, 3H (s), 6.62, 1H (d, *J* = 17.9 Hz), 6.98-7.26,3H (7.08 (d, *J* = 17.9 Hz), 7.18 (ddd, *J* = 0.5, 1.3, 8.0Hz)), 7.37, 2H (ddd, *J* = 0.6, 1.5, 8.0 Hz), 7.41-7.65, 5H (7.49 (ddd, *J* = 0.6, 1.3, 8.0 Hz), 7.55 (ddd, *J* = 0.5, 1.8, 8.0Hz), 7.58 (ddd, *J* = 0.5, 4.7, 8.1 Hz)), 8.06, 1H (ddd, *J* = 1.4, 1.9, 8.1 Hz), 8.57,1H (dt, *J* = 1.9, 4.7 Hz), 9.06,1H (ddd, *J* = 0.5, 1.4, 1.9Hz).

NMR 13C (100 MHz, DMSO*d6*):127.8, 128.8, 129.1, 129.2 (8C; Benzene CH) 130.2, 133.3, 133.5, 140.7 (4C; Benzene-C), 155.6 (1C; Imine), 118.7, 139.0(2C; ethylene CH), 24.3 (1C; Aliphatic CH_3), 163(1C; amide), 125.1, 138.0, 148.2, 153.7 (4C; 3-Pyridine CH), 130.7 (1C; 3-Pyridine C).

CL-5:IR (KBr, cm^{-1}): 3259(N-H), 1574 (C=N), 1520 (C=C aromatic), 1514 (C=C alkenyl), 348 (N=O).

NMR 1H 400 MHz (DMSO-*d*6):δ 2.25,3H (s), 6.88,1H(d, *J* = 17.5 Hz), 7.18-7.33,3H (7.23 (ddd, *J* =0.5, 1.3, 8.0 Hz), 7.29 (d, *J* = 17.5 Hz)), 7.47-7.78, 5H (7.51 (ddd, *J* = 0.5, 1.7, 8.0Hz), 7.59 (ddd, *J* =0.5, 4.7, 8.1 Hz), 7.72 (ddd, *J* = 0.5, 1.9, 8.7Hz)), 8.09,1H (ddd, *J* = 1.4, 1.9, 8.1 Hz), 8.31, 2H (ddd, *J* =0.5,1.8, 8.7 Hz), 8.58, 1H (dt, *J* = 1.9,4.7 Hz), 9.08,1H (ddd, *J* = 0.5, 1.4, 1.9 Hz).

NMR 13C (100 MHz, DMSO*d6*):121.0, 127.3, 129.1, 129.2 (8C; Benzene CH) 130.2, 140.7, 141.3, 147.6 (4C; Benzene-C), 155.6 (1C; Imine), 118.7, 139.0(2C; ethylene CH), 24.3 (1C; Aliphatic CH_3), 163(1C; amide), 125.1, 138.0, 148.2, 153.7 (4C; 3-Pyridine CH), 130.7 (1C; 3-Pyridine C).

CL-6:IR (KBr, cm^{-1}):3265(N-H), 1580 (C=N), 1522 (C=C aromatic), 3084 (Csp2 -H), 2932 (Csp3 –H), 1513 (C=C alkenyl), 742 (C-Cl).

NMR 1H 400 MHz (DMSO-*d*6):δ 1.15, 6H (d, *J* = 7.0 Hz), 2.86,1H (sept, *J* = 7.0 Hz), 6.65,1H (d, *J* = 17.9 Hz), 7.08,1H(d, *J* = 17.9 Hz), 7.28, 2H (ddd, *J* = 0.5, 1.6, 8.0 Hz), 7.36-7.51, 4H (7.42 (ddd, *J* = 0.5, 1.8, 8.6 Hz), 7.44 (ddd, *J* = 0.5, 1.9, 8.0 Hz)), 7.51-7.67, 3H (7.58 (ddd, *J* = 0.5, 4.7, 8.1Hz), 7.62 (ddd, *J* = 0.5, 1.5, 8.6 Hz)), 8.09, 1H (ddd, *J* = 1.4, 1.9, 8.1Hz), 8.57, 1H (dt, *J* = 1.9 ,4.7Hz), 9.06, 1H (ddd, *J* = 0.5, 1.4, 1.9Hz).

NMR 13C (100 MHz, DMSO*d6*):126.1, 129.0, 130.6 (8C; Benzene CH), 131.3, 132.4, 136.6, 147.8 (4C; Benzene-C), 155.6 (1C; Imine), 118.7, 139.0(2C; ethylene CH), 23.4 (2C; Aliphatic CH_3), 36.3 (1C; Aliphatic CH), 163(1C; amide), 125.1,138.0,148.2,153.7 (4C; 3-Pyridine CH), 130.7 (1C; 4-Pyridine C).

3.2 Anti-inflammatory activity

The effect of Chalcone hydrazide derivatives on carrageenan-induced paw edema in rats are summarized in Table 2 and Table 3 and Figure 1. Results showed the significant ($p< 0.001$) increase in paw edema in group-II when compared to vehicle treated control group indicating induction of acute inflammation from 1 hour upto 4 hours.

In present study, chalcone derivative CL-6 showed significant ($p< 0.05$) reduction in carrageenan induced paw edema compared to induction control group from 1 hr to 4 hr where the maximum percentage inhibition 40.30% was noted at 2 hr.The derivative CL-3& CL-1 exhibited significant and equipotent ($p< 0.01$) reduction in carrageenan induced paw edema compared to induction control group from 1 hr to 4 hr and the maximum percentage inhibition 45.62% and 49.42% respectively was recorded at 2 hrs.The carrageenan induced paw edema significantly reduced by chalcone derivative CL-2 treatment when compared to induction control group and reduction was highly significant ($p< 0.001$) and equipotent throughout from 1 hr to 4 hr and the maximum percentage inhibition 61.97% was noted at 2 hrs.The reference standard Indomethacin was effective and significantly ($p< 0.001$) reduced carrageenan induced

paw edema compared to induction control group. The percentage inhibition was found 42.03%, 53.23%, 38.57% and 21.42%at 1 hr,2 hr, 3 hr, and 4 hr respectively.

Table 2: Effect of Chalcone derivatives on carrageenan induced paw edema in rats

Gr. No.	Treatment (n=6)	Paw volume (ml) (Mean±SEM)			
		1 hour	2 hour	3 hour	4 hour
I	Control	0.77 ± 0.6	0.78 ± 0.8	0.77± 0.16	0.77 ± 0.22
II	Induction (Negative Control) Carrageenan (1 % w/v)-	2.26 ± 0.9$^{###}$	2.63 ± 1.31$^{###}$	2.10± 0.61$^{###}$	1.96 ± 0.52$^{###}$
III	Indomethacin - 20mg/kg, p.o	1.31±0.44***	1.23±0.84***	1.29±0.98***	1.54±1.26***
IV	CL-1- 40 mg/kg, p.o.	1.43±0.08**	1.33±0.42**	1.13±0.60**	1.60±0.82**
V	CL-2 **-** 40 mg/kg, p.o.	1.07± 0.18***	1.00 ± 0.32***	0.93 ± 0.42***	1.09 ± 0.6***
VI	CL-3- 40 mg/kg, p.o.	1.57±0.62**	1.43±0.50**	1.42±1.20**	1.82±1.42**
VII	CL-4 – 40 mg/kg, p.o.	1.84± 0.90	1.79±0.83	1.76±0.92	1.91±1.20
VIII	CL-5 - 40 mg/kg, p.o.	1.65± 0.8	1.6 ±0.6	1.63±0.8	1.89±1.30
IX	CL-6 - 40 mg/kg, p.o.	1.60±0.42*	1.57±0.32*	1.55±0.84*	1.85±1.24*

Values are expressesd as Mean ± SEM. # = p<0.05, ##= p<0.01, ### = p<0.001 when compared to control group

*= p<0.05, **=p<0.01, *** = p<0.001 when compared to induction (Negative) control group Statistical significance was analyzed by One-way ANOVA with Dennett's T-test.

Table 3: Effect of Chalcone derivatives on percentage inhibition of edema in carrageenan induced paw edema in rats

Gr. No.	Treatment	% Inhibition of edema (Mean±SEM)			
		1 hour	2 hour	3 hour	4 hour
I	Control	-	-	-	-
II	Induction (Negative Control) Carrageenan (1 % w/v)-	-	-	-	-
III	Indomethacin - 20mg/kg, p.o	42.03	53.23	38.57	21.42
IV	CL-1 - 40 mg/kg, p.o.	36.72	49.42	46.19	18.36
V	CL-2 **-** 40 mg/kg, p.o.	52.65	61.97	55.71	44.38
VI	CL-3 - 40 mg/kg, p.o.	30.53	45.62	32.38	7.14
VII	CL-4 – 40 mg/kg, p.o.	18.58	31.93	16.19	2.55
VIII	CL-5 - 40 mg/kg, p.o.	26.99	38.40	22.38	3.57
IX	CL-6 - 40 mg/kg, p.o.	29.20	40.30	26.90	5.6

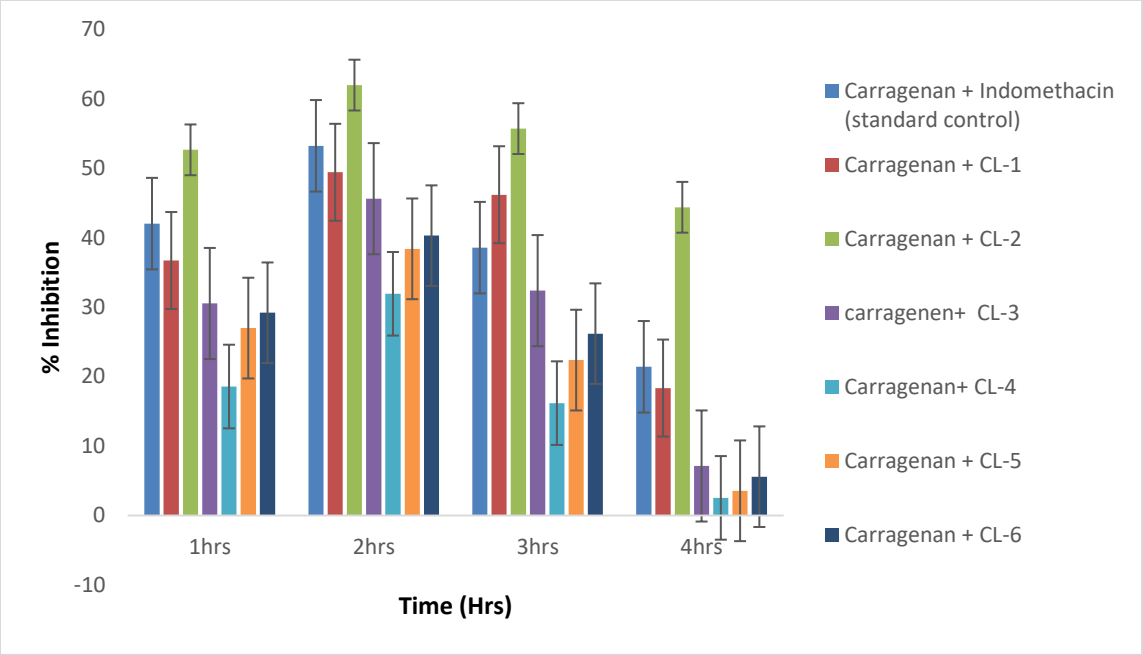


Figure 1: Effect of Chalcone derivatives on percentage inhibition of edema in carrageenan induced paw edema in rats

4. CONCLUSION

In conclusion, Chalcone hydrazide derivatives (CL-1 to CL-6) were synthesized, employing shorter reaction times than the earlier reported in the literature. The spectroscopic characterizations of the six derivatives are in agreement with the structures expected. These chalcones hydrazide derivatives showed an anti-inflammatory protective effect when administered orally route. From the result CL-2 compound (40 mg/kg) showed significant percentage of inhibition against carrageenan induced paw edema which is comparable with reference standard indomethacin.This anti-inflammatory activity was due to presence of electron donating group like $-CH_3$on ring A and electron withdrawing group like NO_2on ring B at 4^{th} position in isonicotinyl hydrazide derivative.

On the basis of result, it can be concluded that chalcone hydrazide derivatives exhibited anti-inflammatory activity. All synthetic chalcone hydrazide derivatives may be considered as safer drugs for treating inflammatory conditions.

CONSENT

The present study did not involve Patients.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

This project was approved by the Animal Ethics Committee from Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune (DYPCOP/IAEC/2021/08).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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