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Analgesic and anti-inflammatory investigation of 1,2,4- triazole derivatives in rats

Neelakanth M Jeedi*, Sandeep Bajji, Pradeepkumar M Ronad, Shrishail K Nimbal, Santosh B Patil

KLE College of Pharmacy, Vidyanagar, Hubballi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India.

Corresponding author: Neelakanth M Jeedi ✉ nmjeedi73@gmail.com, **Orcid Id**: https://orcid.org/0000-0002-7782-2390
Department of Pharmacology, KLE College of Pharmacy, Vidyanagar, Hubballi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India

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ABSTRACT

Pain and inflammation are the major devastating health problems commonly treated with NSAID's. NSAID's are regularly employed to relieve pain and inflammation. However, the 1,2,4-triazole derivatives has been scientifically evaluated for its use relieving pain and inflammation. The current research plan to explore the analgesic and anti-inflammatory property of 1,2,4-triazole derivatives. 1,2,4-trizoles was tested for its anti-inflammatory properties using carrageenan and formalin-induced paw oedema, acetic acid-induced writhing investigation, while its peripheral and central analgesic actions were assessed using the hot plate method and Carrageenan induced Air Pouch model respectively. The test compounds were administrated at dose of 55 mg/Kg. The standard Diclofenac drug was administrated at dose of 10 mg/Kg for Acetic acid induced writhing investigation, Anti-inflammatory activity and 10 mg/Kg dose of pentazocine was administrated for hot plate method. Rats given with distilled water (10 mL/kg) were designated as the negative controls. Oral administration of all treatments was used. Evaluation of the analgesic and anti-inflammatory investigation showed that the second compound (5-(4-nitrophenyl)-1-phenyl-1*H*-1,2,4-triazole-3-(2*H*)–one declines the number of abdominal cramps caused by 1% acetic acid and increases the time of response in hot plate method and decreased the edema induced by carrageenan, formalin also decreased volume of exudates in air pouch model. The results of research indicate that compound 5-(4-nitrophenyl)-1-phenyl-1*H*-1,2,4-triazole-3-(2*H*)-one has shown more effective analgesic and Anti-inflammatory property than the other two compounds.

Keywords: Analgesic, Inflammation, 1,2,4-triazole, Carrageenan, NSAID.

INTRODUCTION

Pain is described as a "disagreeable sensory and emotional involvement connected with existing or impending tissue injury or explained in terms of destruction" by the IASP (International Association for the Study of Pain) [1]. Pain due to tissue damage (distress, inflammation) instigating the discharge of chemical mediators like bradykinin, histamine, serotonin, and prostaglandins they stimulate pain receptors, are able to identify noxious and mild pain in organs [2]. In fact, pain is one of the furthermost collective symptoms that cause patients to seek health care and a chief problem in surgical patients; thus, it should be managed [3,4]. Since numerous pathways work together to produce pain, present analgesic medications continue to have limited efficacy despite an increased understanding of the molecular underpinnings of nociception [5]. Over the previous few years, the only effective compounds have been those targeting either the opioid binding site or the inflammatory pathway. Currently available analgesic medications moderately good and some are producing unfavourable side effects or to be associated with long-period safety concerns [6]. The five cardinal indications of inflammation are redness, swelling (tumour), heat, discomfort and loss of organ function. Inflammation is a defence response to stop or restrict the flow of harmful chemicals. Infectious agents, ischemia, antigen-antibody interactions, injury due to heat or physical damage

are just a few of the many triggers that can cause inflammation [7,8]. The body's initial reaction, which starts in few seconds to minutes after a tissue injury brought on by a damaging stimulus, is acute inflammation. If harmful foreign chemicals persist for a longer period of time and cannot be eliminated by the body, acute inflammation will turn chronic [9]. Similar to how opioids, which are used as potent analgesics, are accompanied by side effects like addiction and dependence, non-steroidal anti-inflammatory drugs are typically used to treat inflammation. However, these drugs are associated with harmful side effects like GI irritation, ulceration, bleeding, etc. [10]. Several tissue nociceptors have been identified that can be activated by noxious heat (Vanilloid receptor 1 and Vanilloid receptor like - 1)[11] or mechanical stimuli (acid sensing ion channels (ASICs)) **[12]**. This study was hence undertaken to evaluate the analgesic and anti-inflammatory activity of newly synthesized 1,2,4-triazole derivatives**.**

MATERIALS AND METHODS

Animals

In this evaluation study adult wistar rats (weighing 150 to 250 g) were used. The animals were housed in cages, 12-hour light/dark cycle in a separate temperature controlled room. Free water and a typical laboratory diet were made available to them. Rats were erratically separated into various groups (six animals in each).

Control group: Normal saline (10 mL/kg)

Standard group: Diclofenac 10 mg/kg

Test compound 1: 55 mg/kg

Test compound 2: 55 mg/kg

Test compound 3: 55 mg/kg

These 1,2,4-triazole derivatives were synthesized in the department of pharmaceutical chemistry, KLE college of pharmacy, Hubballi.

Chemical synthesis protocol

Preparation of N-benzylidene aniline (Schiff base).

Schiff base was synthesized by a mixture of benzaldehyde (2 gm, 0.018 mol) and aniline (2 gm, 0.021 mol) in 25 mL of absolute alcohol in presence of acetic anhydride (0.5 mL 0.0048mol) was refluxed for 6hr in a water bath and allowed to cool. The solvent was removed under pressure. A crude product was obtained which was washed with cold water and was re-crystallized using ethyl actetae and cyclohexane in the ratio 1:2. The purity of the compound was compared by TLC.

Preparation of 1,5-diphenyl-1H-1,2,4-triazole-3-(2H)-one

Schiff base (0.01mol) reacts with urea (0.001mol) in dioxin (25 mL) sin presence of zinc chloride was refluxed for 6hr.after cooling the reaction was poured on crushed ice with stirring. The solid obtained was 1,5-diphenyl-1H-1,2,4-triazole-3-(2H)-one which was then filtered and washed with sodium bicarbonate to remove un-reacted matters and was filtered. The product was dried and re-crystallized from ethanol. % yield 80, M.P R_f value 0.67 solvent system used ethyl acetate: cyclohexane (1:2).

Figure 1: Scheme

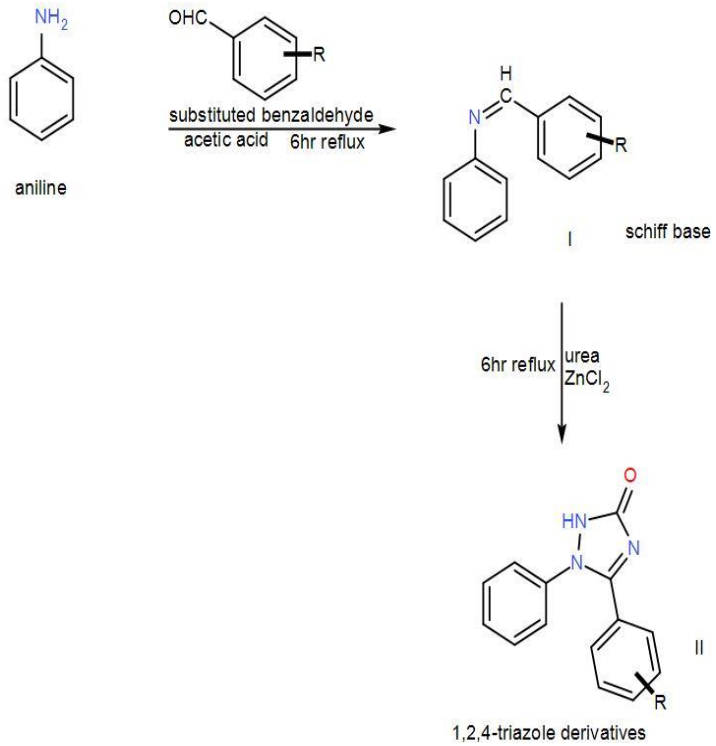


Table 1: Test Compound details

Test Compound	R	% yield	Melting point °C	Molecular weight	Molecular formula	R_f value
1	4-OH	73	160	253.26	$C_{14}H_{11}N_3O_2$	0.62
2	4-NO_2	77.6	176	282.26	$C_{14}H_{10}N_4O_3$	0.64
3	2-Cl	70	162	271.70	$C_{14}H_{10}N_3ClO$	0.75

Chemicals

Distilled water, Diclofenac, Carrageenan (S D Fine-Chem. ltd.), acetic acid (HiMedia Laboratories) and formalin (HiMedia Laboratories) all procured from vendors at Hubballi.

1. Test compound 1; 5-(4-hydroxyphenyl)-1-phenyl-1*H*-1,2,4-triazole-3-(2*H*)-one, (55 mg/kg)
2. Test compound 2; 5-(4-nitrophenyl)-1-phenyl-1*H*-1,2,4-triazole-3-(2*H*)-one, (55 mg/kg)
3. Test compound 3; 5-(2-clorophenyl)-1-phenyl-1*H*-1,2,4-triazole-3-one. (55 mg/kg)

Acute oral toxicity study

Acute oral toxicity studies were carried out as per OECD 425 guidelines [13], on rats of either sex weight 150–200 gm. Approval taken from - Institutional Animal Ethical Committee of KLESCOPH with the following reference number: M.Ph/NC0220007/KLECoPH/21

Analgesic Activity (*Eddy's hot plate)*

Rat population was split into five groups of six animals each. As a control, the group one receive normal saline (10 mL/kg p.o.) and pentazocine (10 mg/kg s.c.) was given to the second group as the

conventional medication. Test compound 1, 2 and 3 (55 mg/kg p.o.) were given to the third, fourth and fifth groups respectively. The device used for the test was Eddy's hot plate instrument [14]. The thermostat was fixed at 55 °C. Rats were located on a hot plate, and reaction times were measured in seconds for jumping or licking the hind paw with a 15-second cut off. After administering the saline vehicle, reference standard drug and test compounds to respective groups, the reaction time was recorded at 0, 30, 60, 90 and 120 minutes.

Acetic acid-induced writhing

The animals divided in group 5 of six in each. The group 1 is control and gets normal saline (10 mL/ kg p.o.). The group 2 animals receive diclofenac sodium (10 mg/kg p.o.) Standard drug and group 3 was administered with test compound 1 (55 mg/kg) group 4 administered with test compound 2 (55 mg/kg) and group 5 was administered with test compound 3 (55 mg/kg). Each rats received an intragastrically administered with vehicle, standard and test compounds respective groups, 60 minutes prior to receiving an 0.6 percent acetic acid solution (10 mL/kg body weight) Intraperitoneal. Following the delivery of acetic acid, each animal's number of writhing reactions was counted over the course of a subsequent 10 minute interval. The writhing inhibition percentage was calculated using below given formula [15].

% Inhibition = (No. of writhing's in Control – No. of writhing's in test) / (No. of writhing's in control) ×100.

Anti-Inflammatory Activity: Carrageenan induced paw oedema

Rats were divided into five groups; each group consisting of six animals. Group 1 control (normal saline 10 mL/kg), Group 2 administered with standard diclofenac (10mg/kg), Group 3 administered with test compound 1 (55 mg/kg), Group 4 administered with test compound 2 (55 mg/kg) and group 5 administered with test compound 3 (55 mg/kg). Paw oedema was brought by sub-plantar injection of 0.1 mL freshly prepared 1% w/v carrageenan suspension into sub-plantar tissue of the left hind paw of each animal an hour after treatment with respective drugs [16]. Using a plethysmometer the volume of the injected paws was measured at 0 and 3 hours after the carrageenan injection.

Myeloperoxidase Assay

Myeloperoxidase enzyme activity in the inflamed ear tissues of various group animals were estimated calorimetrically by measuring absorbance at 630 nm using a Dynatech microplate reader and spectrophotometer. 6 mm ear tissue punch was taken, used to measure enzyme activity. Hexadecyltrimethylammonium bromide (HTAB) is prepared in 0.5% of phosphate buffer, pH 5.4. Following a freeze on dry ice, the sample was kept at -20 ºC until it was tested. The samples were homogenized for 45 seconds at zero degrees in a motor-driven glass/glass homogenizer after being frozen at room temperature for the assay. The vessel was then cleaned with a second 0.75 mL aliquot of HTAB in buffer after homogenate had been decanted into a microfuge tube. The 1.5 mL sample was centrifuged at 12000 x g at 4 for 15 min after the wash was added to the tube. The resulting 30 microliter samples, in triplicate incorporating supernatant into 96-well micro titer plates. The wells were filled with 200 microliters of a solution containing 100 microliters of phosphate buffered saline, 85 microliters of 0.22 M sodium phosphate buffer, pH 5.4, and 15 microliters of 0.017% hydrogen peroxide for the test. Tetramethylbenzidine HC1, 18.4 mM, was added in 20 microliters of 8% aqueous dimethylformamide to start the reaction. The reaction was terminated by adding 30 microliter of 1.46 M sodium acetate, pH 3.0, to each well of the plates after they had been incubated at 37 ºC for 3 min. absorbance noted at 630 nm [17].

Granuloma Pouch Model

Rats that have been with 24 hr fast and unrestricted access to water were employed. The respective group animals received vehicle, standard drug and test compounds. The animal's back is shaved and cleaned an hour after dosage. In order to prevent any air leakage, 4 mL of 2% carrageenan in normal saline was given into the subcutaneous dorsal granuloma pouch created in the ether-anesthetized rats after injecting 6 mL of air. The procedure was repeated daily for seven days. On day 8 the pouch is opened under anesthesia and the exudate volume was gathered using a syringe. The weights of granulomas, total WBC count and average exudate volume are noted [18].

Formalin induced paw edema

Rats were divided into 5 groups; each group consisting of six animals. Normal saline, standard drug and test compounds 1, 2 and 3 were given to respective group animals. One hour later paw edema was induced by injecting 0.2 mL of 2% v/v freshly prepared formalin solution prepared in distilled water into sub-plantar tissues of the left hind paw of each rat. The oedema volume was measured before and after injecting the formalin every day at a fixed time for seven consecutive days using a plethysmometer [19].

Statistical analysis

One-way ANOVA (Analysis of Variance) followed by Dunnett's multiple comparison tests were performed to assess the findings of the current study. Data was calculated using Graph Pad Prism 5 programme for statistical analysis. The results expressed as Mean ± SEM (Standard Error of Mean). *p* value less than 0.001 considered highly significant, if *p* value more than 0.001 and less than 0.01 considered moderately significant, if *p* value more than 0.01 and less than 0.05 considered weakly significant, if *p* value more than 0.05 considered non-significant.

RESULTS AND DISCUSSION

Acute Oral Toxicity Study

Following up and down method of dose administration, it

has been found that three test compounds do not produce any toxic effects at the maximum dose of 550 mg/kg body weight. Hence one-tenth of the lethal dose was considered as the therapeutic dose of 1,2,4-triazole derivatives used for pharmacological activity was 55 mg/kg body weight.

Analgesic activity

Eddy's hot plate method

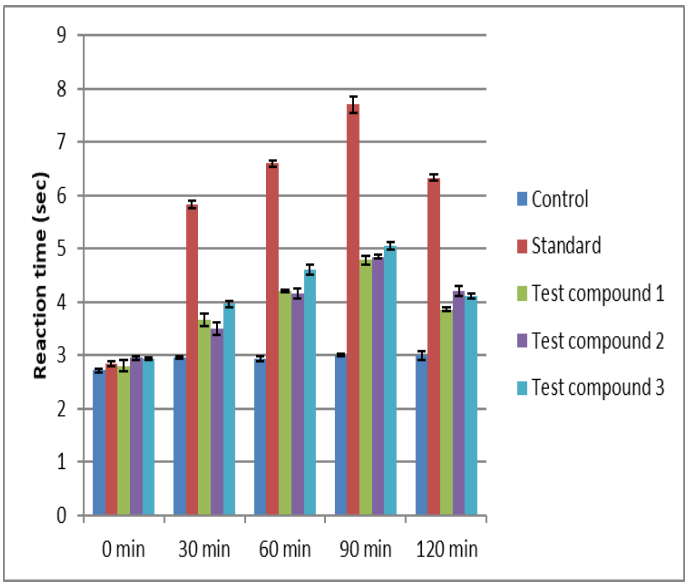
In the control group the rats have shown the normal response i.e., jumping and paw licking or withstand on the hot plate. The standard drug pentazocine (10 mg/kg) treated group animals showed significant ($P < 0.001$) increase in jumping and paw licking responses latency compare to control group animals, it shows better result at 90 min. Test compounds treated group animals showed increase in response time compare to control group animals. Among the three test compounds, the test compound 2; 5-(4-nitrophenyl)-1-phenyl_1*H*-1,2,4-triazole-3-(2*H*)-one showed highest increases ($P < 0.001$) in response time also showed better results at 90 min. compare to control group animals. The analgesic effectiveness of compound 2 at 55 mg/kg was comparable to standard pentazocine. (Table 1, Figure 1)

Table 1: Effect of 1,2,4 -triazole derivatives on Hot plate test in rats

Group	Dose (mg/kg, B.W.)	Before treatment	After Treatment			
			30 min.	60 Min.	90 Min.	120 Min.
Control (NS)	10 mL/kg	2.71±0.03	2.96±0.03	2.93±0.04	3.0±0.02	3.0±0.08
Standard (Pentazocine)	10 mg/kg	2.83±0.08	5.83±0.07***	6.6±0.06***	7.7±0.06***	6.33±0.03***
Test compound1	55 mg/kg	2.8±0.06	3.66±0.06	4.2±0.07***	4.78±0.08**	3.86±0.04**
Test compound 2	55 mg/kg	2.95±0.08	3.5±0.08**	4.16±0.08***	4.85±0.01***	4.2±0.09***
Test compound 3	55 mg/kg	2.93±0.05	3.96±0.06	4.61±0.07***	5.06±0.07**	4.11±0.05**

*ANOVA followed by Dennett's test was performed. Each value is the Mean ± Standard error of mean (SEM). NS: Normal saline. Statistically significant values: * significant p < 0.05, ** significant p < 0.01, *** significant p < 0.001.*

Figure 1: Effect of 1,2,4-traizole derivative on Eddy's hot plate method



Acetic acid induced writhing

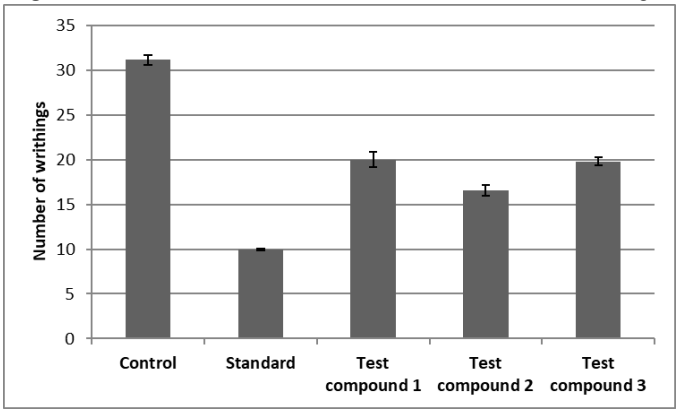
The normal control group animals showed maximum number of writhes. The number of writhing's response were significantly ($P < 0.001$) reduced in the diclofenac (10 mg/kg) treated group animals compared to normal control. In the test compounds treated group animals also reduction in number of writhes were witnessed. Among the three test compounds, the test compound 2: 5-(4-nitrophenyl)-1-phenyl_1*H*-1,2,4-triazole-3-(2*H*)-one significantly ($P < 0.001$) reduces number of writhing's and stretching's. The test compound 2: 5-(4-nitrophenyl)-1-phenyl_1*H*-1,2,4-triazole-3-(2*H*)-one showed maximum percentage of inhibition (46%) of writhing's compare to other test compounds 1 (35%) and 3 (36%). (Table 2, Figure 2)

Table 2: Effect of 1,2,4 -triazole derivatives on acetic acid induced writhing's

Group	Dose	Number of writhing	Percentage inhibition
Control (NS)	10 mL/kg	31.16±0.54	00.00
Standard (Diclofenac)	10 mg/kg	09.96±0.06***	70.60
Test compound 1	55 mg/kg	20.00±0.84**	35.81
Test compound 2	55 mg/kg	16.60±0.58***	46.72
Test compound 3	55 mg/kg	19.83±0.49**	36.36

*ANOVA followed by Dennett's test was performed. Each value is the Mean ± SEM. NS: Normal saline. Statistically significant values: * significant p < 0.05, ** significant p < 0.01, *** significant p < 0.001.*

Figure 2: Effect 1,2,4 -triazole derivatives on acetic acid induced writhing's



Anti-inflammatory Activity: Carrageenan Induced Paw oedema

It was observed that, the control group injected with carrageenan caused localised oedema started at 1 hr after injection, the swelling increased progressively to the maximum volume at 4 hr after carrageenan injection. Standard diclofenac pre-treated group animals showed significant ($p < 0.001$) inhibition of inflammation at 1st, 2nd, 3rd and 4th hr after administration, when compared to normal control group animals. All three test compounds pre-treated respective group animals showed moderate ($p < 0.01$) reduction in

paw oedema at 1st hr but significant ($p < 0.001$) reduction of oedema formation induced by carrageenan at 2nd, 3rd and 4th hr The test compound 1 and 3 has same ability and compound 2 has more ability of reduction of oedema induced by carrageenan. (Table 3, Figure 3 & 4).

Table 3: Effect of 1,2,4 -triazole derivatives on carrageenan induced paw oedema

Group	Dose	Before Treatment	After treatment			
			1 hr	2 hr	3 hr	4 hr
Normal (NS)	10 mL/kg	0.85±0.03	1.44±0.04	1.68±0.07	1.91±0.05	1.97±0.05
Standard (Diclofenac)	10 mg/kg	0.85±0.02	1.01±0.01***	1.13±0.02***	1.10±0.02***	1.04±0.02***
Test Compound 1	55 mg/kg	0.853±0.021	0.880±0.15**	1.17±0.01***	1.32±0.02***	1.29±0.02***
Test Compound 2	55 mg/kg	0.878±0.01	1.06±0.01**	1.18±0.01***	1.31±0.008***	1.25±0.01***
Test Compound 3	55 mg/kg	0.895±0.01	1.037±0.04**	1.14±0.08***	1.28±0.04***	1.29±0.04***

*ANOVA followed by Dennett's test was performed. Each value is the Mean ± SEM. NS: Normal saline. Statistically significant values: * significant $p < 0.05$, ** significant $p < 0.01$, *** significant $p < 0.001$.*

Figure 3: Effect 1,2,4 -triazole derivatives on carrageenan induced paw oedema

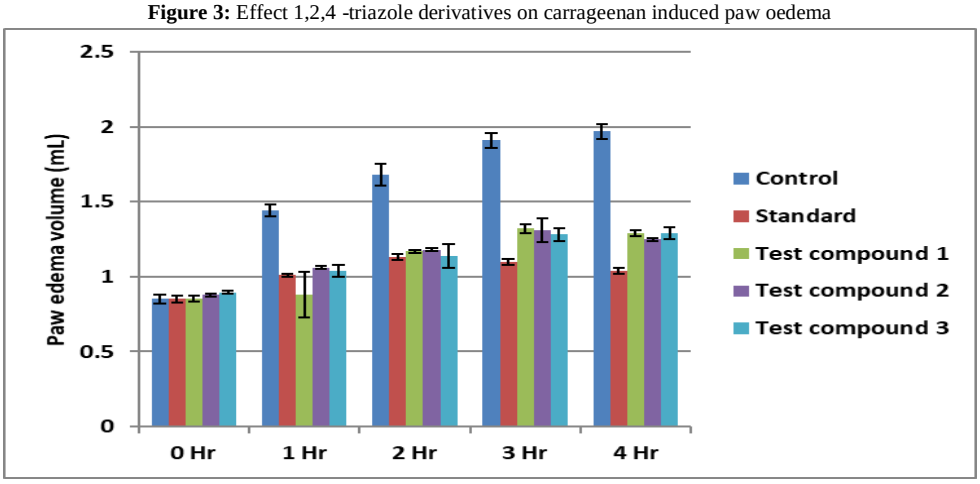
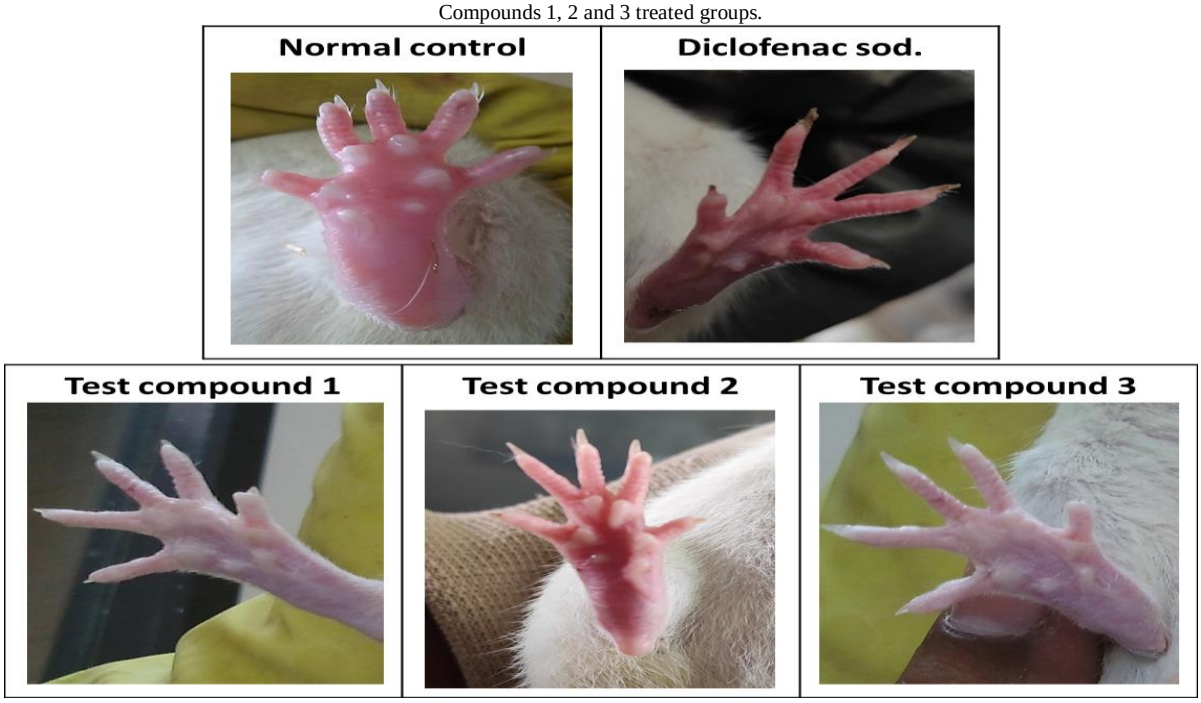


Figure 4: Carrageenan-induced hindpaw oedema model for assessment of Anti-inflammatory activity. Normal Control, Standard Diclofenac treated, and Test Compounds 1, 2 and 3 treated groups.



Granuloma pouch

It was observed that, the volume of lavage fluid was more in granuloma pouch of control group animals when compared to control. Standard drug diclofenac given animals showed significant ($P < 0.001$) reduction in exudative volume or lavage fluid volume (37% inhibition) in granuloma pouch when compared to normal control group. All three test compounds also reduce the exudative volume in granuloma pouch compare to normal control group, but vary in their ability. Test compound 3 has slightly reducing ($P < 0.05$) (17% inhibition) ability, test compound 1 has moderately reducing ($P < 0.01$) (31% inhibition) ability and test compound 2 has highly reducing ($P < 0.001$) ability of exudative volume or lavage fluid volume (33% inhibition). (Table 4, Figure 5)

Table 4: Effect of 1,2,4 -triazole derivatives on volume of exudates in granuloma pouch model

Group	Dose	Volume of Lavage Fluid (mL)	% Inhibition
Normal	10 mL/kg	3.5±0.09	00.00
Standard (Diclofenac)	10 mg/kg	2.2±0.12***	37.14
Test compound 1	55 mg/kg	2.48±0.1**	31.42
Test compound 2	55 mg/kg	2.33±0.14***	33.42
Test compound 3	55 mg/kg	2.88±0.13*	17.71

*ANOVA followed by Dennett's test was performed. Each value is the Mean ± SEM. NS: Normal saline. Statistically significant values: * significant p < 0.05, ** significant p < 0.01, *** significant p < 0.001.*

Figure 5: Effect of 1,2,4 -triazole derivatives on volume of exudates in granuloma pouch model

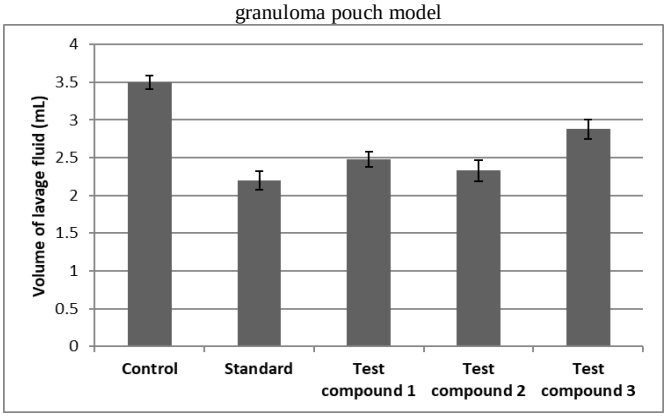


Table 5: Effect of 1,2,4 -triazole derivatives on formalin induced paw oedema

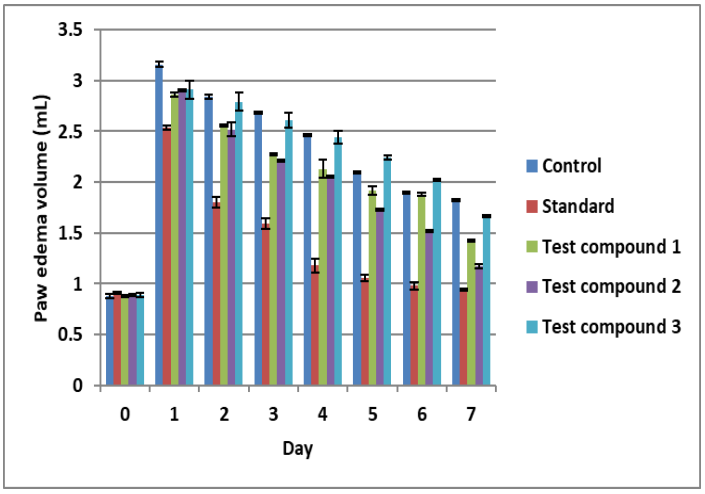
Group	Dose	Before induction	After treatment						
Days		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control (NS)	10 mL/kg	0.88±0.02	3.16±0.03	2.84±0.02***	2.68±0.01***	2.46±0.01	2.10±0.01	1.90±0.01	1.82±0.01
Standard (Diclofenac)	10 mg/kg	0.91±0.01	2.54±0.02***	1.80±0.05***	1.59±0.05***	1.18±0.07***	1.06±0.03***	0.98±0.04***	0.94±0.01***
Test Compound 1	55 mg/kg	0.88±0.01	2.86±0.02*	2.56±0.01*	2.27±0.01*	2.13±0.09*	1.92±0.04**	1.88±0.02**	1.42±0.01**
Test Compound 2	55 mg/kg	0.89±0.01	2.9±0.01**	2.52±0.07**	2.21±0.01**	2.05±0.01***	1.73±0.01***	1.52±0.01***	1.17±0.02***
Test Compound 3	55 mg/kg	0.89±0.02	2.91±0.09*	2.79±0.09*	2.61±0.07*	2.44±0.06*	2.24±0.02*	2.02±0.01*	1.67±0.01*

*ANOVA followed by Dennett's test was performed. Each value is the Mean ± SEM. NS: Normal saline. Statistically significant values: * significant p < 0.05, ** significant p < 0.01, *** significant p < 0.001*

Formalin induced paw oedema

The formalin (0.1 mL of 2%) received normal control group animals showed maximum paw volume on day 1 but slight decrease in paw oedema from day 2 to day 7, at the end of day 7 also oedema persist. In the standard drug diclofenac treated group animals the paw volume was significantly ($P < 0.001$) decreases and goes on decreasing day by day, on day 7 paw volume was almost near to before induction. The test compound 1, 2 and 3 treated group animals also showed significant ($P < 0.01$, $P < 0.001$ and $P < 0.05$ respectively) reduction in paw volume when compared to before induction. The test compound 2 has better anti-inflammatory activity compare to other two compounds and results were comparable to standard drug. (Table 5, Figure 6)

Figure 6: Effect of 1,2,4 -triazole derivatives on formalin induced paw oedema



Total WBC Count

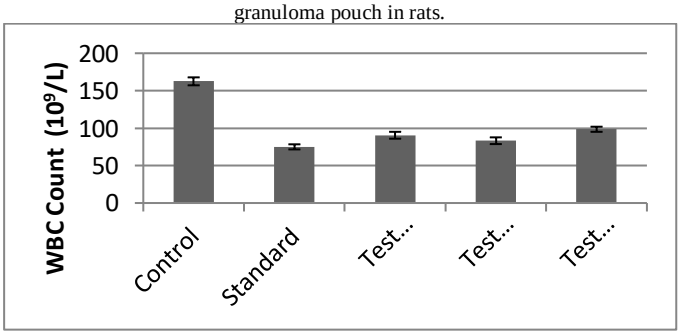
In the exudate of granuloma pouch of normal control group animals, the total WBC count was very high as compare with that of standard drug treated group. Standard diclofenac treatment group showed significantly ($P < 0.001$) reduction in the number of total WBC count when compared with normal control. The test compounds 1, 2 and 3 treatment significantly ($P < 0.01$, $P < 0.001$ and $P < 0.01$ respectively) reduces the total WBC count in the exudate of granuloma pouch in the respective group animals when compared to normal control group. (Table 6, Figure 7)

Table 6: Effect of 1,2,4 -triazole derivatives on WBC Count in exudate of granuloma pouch in rats.

Groups	Dose	WBC (10^9/L)
Control (NS)	10 mL/kg	162.5±5.3
Standard (Diclofenac)	10 mg/kg	75.0±3.4***
Test compound 1	55 mg/kg	90.5±4.6**
Test compound 2	55 mg/kg	83.2±4.5***
Test compound 3	55 mg/kg	98.5±3.4**

*ANOVA followed by Dennett's test was performed. Each value is the Mean ± SEM. NS: Normal saline. Statistically significant values: * significant p < 0.05, ** significant p < 0.01, *** significant p < 0.001.*

Figure 7: Effect of 1,2,4 -triazole derivatives on WBC Count in exudate of granuloma pouch in rats.



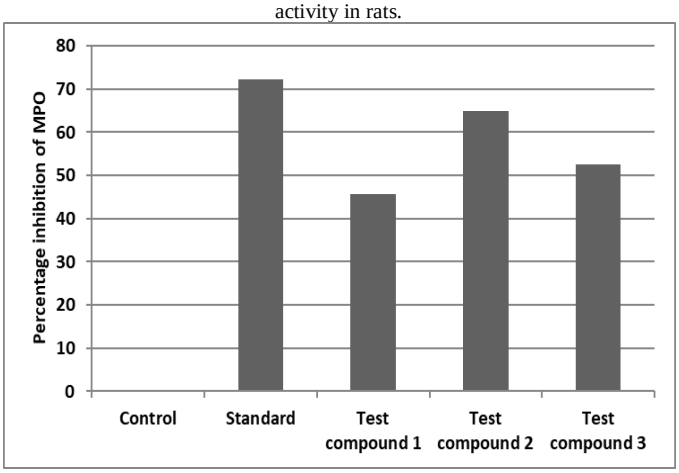
Myeloperoxidase

The myeloperoxidase enzyme activity is very high in the inflamed ear tissue (TPA-tetradecanoyl phorbol acetate induced) of control group animals. The standard drug showed (72.21%) reduction in the level of MPO enzyme activity in the homogenates of the inflamed tissues. The test compounds 1, 2 and 3 treated groups also showed 45.62%, 64.93% and 52.43% reduction in the myeloperoxidase enzyme activity respectively. Among three test compounds test compound 2 showed maximum reduction of myeloperoxidase enzyme activity. (Table 7, Figure 8)

Table 7: Effect of 1,2,4 -triazole derivatives on Myeloperoxidase enzyme activity in rats.

Groups	Dose	% Inhibition of MPO
Control (NS)	10 mL/kg	00.00
Standard (Diclofenac)	10 mg/kg	72.21
Test compound 1	55 mg/kg	45.62
Test compound 2	55 mg/kg	64.93
Test compound 3	55 mg/kg	52.46

Figure 8: Effect of 1,2,4 -triazole derivatives on Myeloperoxidase enzyme activity in rats.



Interleukin-6 (IL-6)

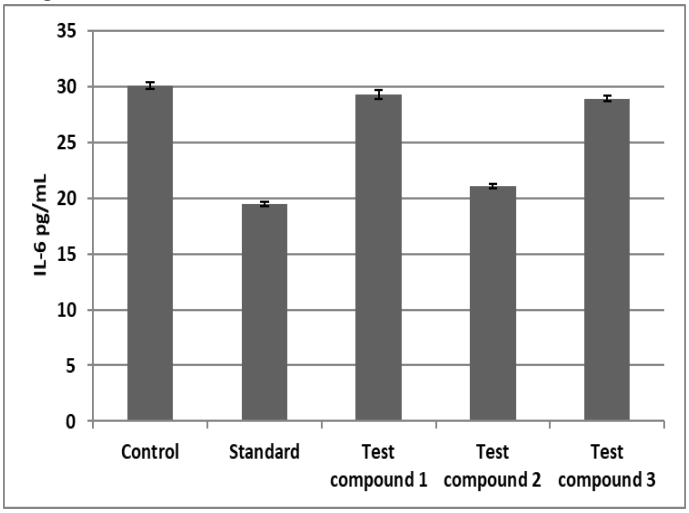
In the carrageenan induced paw edema of normal control group animals the IL-6 level was very high as compare with that of standard drug treated group. Standard diclofenac treatment group showed significantly ($P < 0.001$) reduction in the IL-6 level when compared with normal control. The test compounds 1, 2 and 3 treatments significantly ($P < 0.05$, $P < 0.001$ and $P < 0.05$ respectively) reduces the IL-6 levels in respective groups when compared to control group. (Table 8, Figure 9)

Table 8: Effect of 1,2,4 -triazole derivatives on interleukins level in rats.

Groups	Dose	IL-6 pg/mL
Control (NS)	10 mL/kg	30.15±0.3
Standard (Diclofenac)	10 mg/kg	19.52±0.2***
Test compound 1	55 mg/kg	29.30±0.4*
Test compound 2	55 mg/kg	21.12±0.2***
Test compound 3	55 mg/kg	28.95±0.3*

*ANOVA followed by Dennett's test was performed. Each value is the Mean ± SEM. NS: Normal saline. Statistically significant values: * significant p < 0.05, ** significant p < 0.01, *** significant p < 0.001.*

Figure 9: Effect of 1,2,4 -triazole derivatives on interleukins level in rats.



Identifying safer and more effective analgesic and anti-inflammatory drugs required to minimize the usage of present available drugs with more side effects [20]. The current study, which was conducted to assess the analgesic and anti-inflammatory property of newly synthesized three compounds i.e., 1,2,4-triazole derivatives. One among three 1,2,4-triazole derivatives showed potent anti-analgesic and anti-inflammatory activity, in various nociception and Inflammatory animal models.

Nociception is the mechanism; whereby noxious peripheral stimuli are transmitted to brain. Nociceptive strands end in the shallow layers of the dorsal horn, shaping synaptic associations with transmission neurons rushing to the thalamus [21]. Nociceptors release glutamate, substance P contributing to neurogenic inflammation. 1,2,4-triazole derivatives showed analgesic effect possibly because of decrease in nociceptors sensitivity, blocking voltage gated ion channels. 1,2,4-triazole derivatives inhibit impulse generation and conduction by sensory nerves through dorsal route ganglia, acting through GABA receptors, inhibit release of glutamate centrally or by both the mechanisms.

Acetic acid induced writhing is a response to the inflammation and intense pain induced by irritant principles via nociceptors activation, characterized by episodes of retraction of abdomen and stretching of hind limbs [22]. The signals transmitted to central nervous system in response to pain due to irritation. The analgesic property of 1,2,4-triazole derivatives because they stabilizes the cells for response to acetic acid and reduces the release of mediators such as prostaglandins, bradykinin and histamine.

Accumulation of IL-6, prostaglandin, neutrophil, TNF-α, myeloperoxidase and prostaglandins is important characteristic of inflammatory condition [23]. Indeed, an ability to estimate the quantity of il-6, the severity of inflammation is identified by neutrophils levels in inflamed tissue or the effect of experimental or

therapeutic operate to alter the inflammatory response.

Aggravation prompted via carrageenan is intense, non-resistant, well-informed and profoundly reproducible. Cardinal signs of inflammation are oedema; hyperalgesia and erythema develop immediately following subcutaneous injection, resulting from action of pro-inflammatory agents like bradykinin, histamine, IL-6, reactive oxygen and nitrogen species. Such chemicals can be produced *in situ* at the site of affront or by penetrating cells. The 1,2,4-triazole derivatives stabilize the cells from responding to carrageenan and thus reduces the secretions of inflammatory mediators, namely prostaglandins, bradykinin, histamine, IL-6, myeloperoxidase, reactive oxygen and nitrogen species. It is clearly confirmed that 1,2,4-triazole derivatives treatment significantly decreases the levels of inflammatory biomarkers like IL-6 and myeloperoxidase. The granuloma air pouch model is characterized as local and chronic inflammation [24]. The 1,2,4-triazole derivatives reduces the fluid exudation and accumulation of blood cells into granuloma air pouch. It is clearly confirmed that 1,2,4-triazole derivatives treatment significantly decreases the fluid level and WBC count in granuloma air pouch. Onset of oedema because of formalin is basically a result of neurogenic irritation mediated by neuropeptides like substance P [25]. Higher doses of formalin aggravate the release of 5-hydroxytryptamine, histamine, proteinoids along with substance P. Antioxidant compounds have been known to show anti-inflammatory properties [26,27]. The tested 1,2,4-triazole derivatives reduces the oedema formation induced by formalin. It is evidently confirmed that 1,2,4-triazole derivatives treatment significantly decreases the oedema level by inhibiting the levels of neuropeptides, substance P, 5-hydroxytryptamine, histamine and proteinoids.

CONCLUSION

The study demonstrated that the test compound 2, i.e., 5-(4-nitrophenyl)-1-phenyl-1*H*-1,2,4-triazole-3-(2*H*)-one, showed superior analgesic and anti-inflammatory activity at dose of 55 mg/kg compared to the rest of test compounds.

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Conflict of interest

Authors declared that we don't have any conflict of interest.

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