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Research Article

STUDY OF ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITY OF NOVEL ISATIN DERIVATIVES

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ABSTRACT

Isatin is a endogenous compound and it has a wide variety of pharmacological activities. In the present investigation a different isatin derivatives such as Isatin-3-[N[2]-(2-benzalaminothiazol-4-yl)]hydrazones were taken (IA-IJ) and their anti-inflammatory, analgesic and antipyretic activity was evaluated in animal models with a doses of 10 mg/kg and 100 mg/kg as a test doses using indomethacin (10 mg/kg) as a standard. Anti-inflammatory activity by carrageenan induced rat paw edema model in which mean increase in paw volume and % inhibition of paw volume was measured with plethysmometer at different time intervals. Antipyretic activity was evaluated by Brewer's yeast induced pyresis model and measured reduction of rectal temperature. Eddy's hot plate method was employed for analgesic activity. All the compounds were screened, among the screened compounds ID, IF, IH and IJ showed anti-inflammatory, analgesic and antipyretic activity with ($p<0.001$) dose dependent effect when compared with the control group.

Keywords: Isatin, hydrazones, anti-inflammatory, analgesic and antipyretic activity.

INTRODUCTION

Isatin is an endogenous compound identified in humans[1]and isolated in 1988 and reported to possess a wide range of activities.[2]Isatins have been used as valuable synthetic intermediates in both pharmaceutical and dye industries for many decades.[3] Isatin was first obtained by Erdman[4] and Laurent[5] in 1841 as a product from the oxidation of indigo by nitric and chromic acids. Isatins are also used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines and as raw materials for drug synthesis.[6] A comprehensive literature survey revealed that isatin possess diverse chemotherapeutic activities such as antibacterial,[7] antifungal,[7] anti-HIV,[7] anti-mycobacterial,[8] anticancer,[9] anti-inflammatory,[10]analgesic,[10]anticonvulsant,[11]Diuretic,[12] H_1-anti-hisat-aminic activity,[13] antidepressant,[13] MAO Inhibitor,[14] and antioxidant activity[15] etc.

MATERIALS AND METHODS

Chemicals

Indomethacin gifted by Microlabs, Bangalore, Carrageenan, Brewer's yeast from Sigma-Aldrich Chemicals, USA and all other chemicals & reagents were used analytical grade.

Animals

Wistar albino rats (150-200 g) of age between 8-12 weeks and Swiss albino mice (20-30 g) of age 3-4 weeks of either sex procured from Mahaveer enterprises, Hyderabad, India. The animals were housed under standard laboratory conditions maintained at 25±1 °C and under 12/12 h light/dark cycle and fed with standard pellet diet and water *ad libitum*. The animal experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC NO: 1047/ac/07/ CPCSEA).

Anti-inflammatory Activity

The Wistar strain of albino rats weighing 150-200 gm were taken and they were divided into different groups (n=6) were fasted for 12 hours prior to induction of edema although water was available *ad libitum*. Rats were deprived of water only during the experiment to ensure uniform hydration and minimize any variability in edematous response. Inflammation of the hind paw was induced by injecting 0.1 ml of 1 % carrageenan in normal saline into the sub plantar region of the right hind paw .The control group received only 0.1% sodium (carboxy methyl cellulose) CMC and the standard group received indomethacin (*p.o,* 10mg/kg). The test groups received test compounds at doses of 10 and 100 mg/kg, through *p.o* route. All the sample treatments were given 1 hr before the carrageenan injection; edema was expressed as the increase in paw volume. The paw volume was measured with a digital Plethysmometer (UgoBasile, 7140) before and 1, 2, 3, and 4 hr after the carrageenan injection. Percentage of paw volume inhibition was calculated from the following formula[16]

$$\text{Percent inhibition} = (PC - PT)\ /PC \times 100$$

Where PC and PT is the increase in paw volume in control and test respectively.

Analgesic Activity

Adult male or female mice weighing 20-30 gm were used and they were fasted overnight and divided into different groups (n=6) and the experiment were carried out using Eddy's hot plate method.[17] The temperature was set at 55±1 °C and mice were placed on a hot plate and recorded the reaction time in seconds for licking of hind paw or jumping with cut-off time of 15 secs. Following the administration of the test compounds (10 and100 mg/kg), standard indomethacin (10 mg/kg) and control 0.1% sodium CMC were administered orally, all animals reaction time was recorded at 0, 30, 90 and 120 min.

Anti-pyretic Activity

Wistar strain rats of either sex weighing 150-200 gm were taken and animals were fasted overnight and divided into different groups (n=6). Fever was induced by injecting 20 ml/kg (*s.c*) of 20% suspension of Brewer's yeast in normal saline below the nape of the neck and the initial rectal temperature was recorded. After 18h, animals that showed an increase of 0.3-0.5 °C in rectal temperature were selected for the experiment. The test compounds (10 and 100 mg/kg), standard indomethacin (10 mg/kg) and control 0.1% sodium CMC were administered orally. The rectal temperature was measured 30 min before and at 30, 60, 90 and 120 min after compounds administration.[18]

Statistical analysis

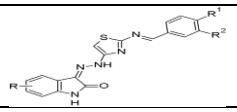
Results were expressed as Mean ± SD, statistical significance was calculated by applying one way ANOVA. $P<0.05$ was considered as significant (Newman-Keuls multiple comparison test).

RESULTS

Carrageenan-induced Pawedema

Anti-inflammatory effect of Isatin-3-[N²-(2-benzalaminothiazol-4-yl)] hydrazones derivatives (IA-IJ) were shown in Table 2. From the observations ID, IF, IH and IJ have significant (P<0.05-0.001) anti-inflammatory activity when compared with the control. The % inhibition in paw edema after 3 h were recorded as 53.70 for indomethacin and 31.48, 31.48, 38.88 and 44.44 for ID, IF, IH and IJ respectively with 100mg/kg.

Table 1: Physical data of Isatin-3-[N²-(2-benzalaminothiazol-4-yl)]hydrazones



Compound	R	R¹	R²	MF	MW
IA	H	H	H	$C_{18}H_{13}N_5OS$	347
IB	H	Cl	H	$C_{18}H_{12}ClN_5OS$	381
IC	H	$N(CH_3)_2$	H	$C_{20}H_{18}N_6OS$	390
ID	H	OH	OCH_3	$C_{19}H_{15}N_5O_3S$	393
IE	5-CH_3	Cl	H	$C_{19}H_{14}ClN_5OS$	395
IF	5-CH_3	OH	OCH_3	$C_{20}H_{17}N_5O_3S$	407
IG	5-CH_3	H	H	$C_{19}H_{15}N_5OS$	361
IH	5-Cl	OH	OCH_3	$C_{19}H_{14}ClN_5O_3S$	427
II	5-Cl	Cl	H	$C_{18}H_{11}Cl_2N_5OS$	416
IJ	5-NO_2	OH	OCH_3	$C_{19}H_{14}N_6O_5S$	438

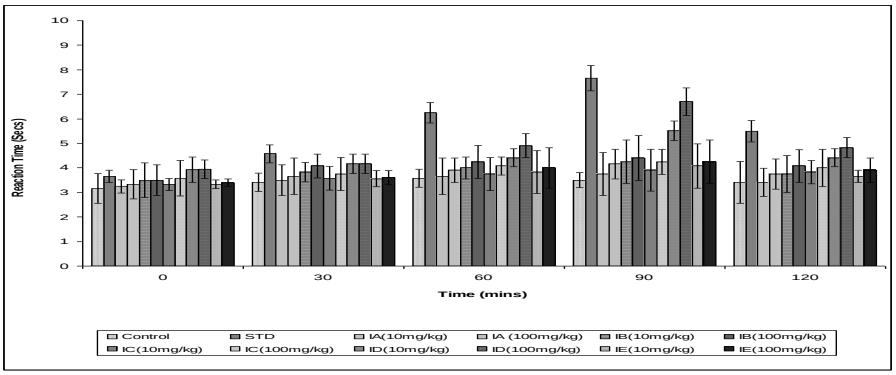
Table 2:Anti-Inflammatory Activity of Isatin-3-[N²-(2-Benzalaminothiazol-4-yl)]hydrazones by Carageenan Induced Paw Edema in rats.

Treatment	Dose (mg/kg)	Mean paw volume in ml (% inhibition)			
		1hr	2hr	3hr	4hr
Control	------	0.31±0 .07	0.41±0.07	0.65±0.05	0.45±0.05
STD	10	0.28±0.07(10.52)	0.31±0.05*(24.00)	0.38±0.07*** (41.02)	0.20±0.04***(53.70)
IA	10	0.30±0.06(5.26)	0.38±0.09(8.00)	0.59±0.02(8.97)	0.40±0.06(9.25)
	100	0.29±0.04(7.89)	0.37±0.03(9.2)	0.58±0.04(9.74)	0.40±0.07(11.11)
IB	10	0.30±0.04(2.63)	0.39±0.02(6.00)	0.59±0.02(8.90)	0.39±0.06(11.85)
	100	0.29±0.02(7.36)	0.38±0.04(8.00)	0.58±0.02(10.25)	0.39±0.08(12.22)
IC	10	0.30±0.04(2.63)	0.39±0.02(6.00)	0.59±0.03(8.20)	0.40±0.04(9.25)
	100	0.29±0.02(6.84)	0.38±0.04(8.00)	0.58±0.02(9.48)	0.40±0.05(11.11)
ID	10	0.29±0.03(6.31)	0.36±0.04(12.00)	0.55±0.05*(15.38)	0.35±0.05*(22.22)
	100	0.29±0.02(7.89)	0.35±0.04(14.00)	0.50±0.04***(21.79)	0.30±0.02***(31.48)
IE	10	0.30±0.04(2.63)	0.40±0.03(4.00)	0.60±0.02(6.41)	0.41±0.04(7.40)
	100	0.30±0.06(5.26)	0.39±0.04(5.6)	0.59±0.02(8.94)	0.40±0.02(9.25)
IF	10	0.29±0.05(6.84)	0.37±0.04(10.00)	0.54±0.04*(16.66)	0.34±0.04*(24.07)
	100	0.29±0.03(8.42)	0.36±0.06(12.00)	0.49±0.06***(24.35)	0.30±0.02***(31.48)
IG	10	0.30±0.04(2.63)	0.39±0.02(6.00)	0.59±0.02(8.97)	0.40±0.06(9.25)
	100	0.30±0.06(5.26)	0.38±0.04(8.00)	0.57±0.06(11.53)	0.39±0.06(11.81)
IH	10	0.29±0.07(6.31)	0.36±0.04(12.00)	0.52±0.06**(19.23)	0.29±0.02***(35.18)
	100	0.29±0.04(7.89)	0.35±0.04(15.20)	0.46±0.05***(28.20)	0.27±0.04***(38.88)
II	10	0.30±0.02(2.63)	0.40±0.06(4.00)	0.61±0.04(5.12)	0.41±0.02(7.40)
	100	0.30±0.03(4.21)	0.39±0.02(6.00)	0.60±0.06(7.69)	0.41±0.04(8.14)
IJ	10	0.30±0.03(5.26)	0.35±0.04(14.00)	0.52±0.04**(19.74)	0.26±0.05***(40.74)
	100	0.29±0.04(7.89)	0.34±0.04(16.40)	0.45±0.04***(30.76)	0.25±0.04***(44.44)

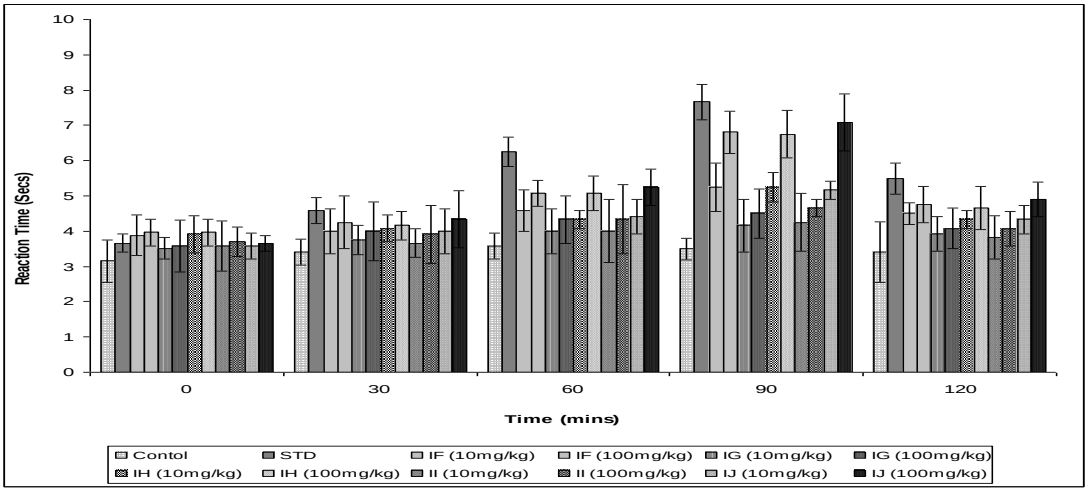
Values are Mean±SD, n=6 in each group.*P<0.05, **P<0.01, ***P<0.001 when compared with control group (Newman-Keuls multiple comparison test)

Analgesic activity determined using Eddy's hot plate method

The analgesic activity of Isatin-3-[N²-(2-benzalaminothiazol-4-yl)] hydrazones derivatives (IA-IJ) and its significant (P<0.05-0.001) were shown in Graph 1 and 2. From the data ID, IF, IH and IJ were exhibited marked central analgesic effect as evidenced by significant increase in reaction time when compared to the control.



Graph 1: Analgesic Activity of Isatin-3-[N²-(2-benzalaminothiazol-4-yl)] hydrazones (IA-IE) by Eddy's hot plate method. N=6, values are expressed as mean±SD, p<0.05 when compared with control.



Graph 2: Analgesic Activity of Isatin-3-[N²-(2-benzalaminothiazol-4-yl)] hydrazones (IF-IJ) by Eddy's hot plate method. N=6, values are expressed as mean±SD, p<0.05 when compared with control.

Brewer's yeast induced pyrexia

The result of the effect of the Isatin-3-[N²-(2-benzalaminothiazol-4-yl)] hydrazones derivatives (IA-IJ) were against Brewer's yeast induced pyrexia. There was a progressive dose dependent reduction in the temperature of rats treated with the compounds ID, IF, IH and IJ. The test of significance (P<0.005-0.001) of the compounds calculated by comparing with control and represented in Table 3.

Table 3. Effect of Isatin-3-[N²-(2-Benzalaminothiazol-4-yl)] hydrazones by Brewer's Yeast induced Hyperthermia in rats.

Treatment	Dose (mg/kg)	Rectal temperature (◦c)					
		Before yeast	18 hr after yeast	Time after treatment (min)			
				30	60	90	120
Control	------	38.10±0.20	38.61±0.27	38.71±0.27	38.83±0.38	38.75±0.49	38.45±0.48
STD	10	38.05±0.15	38.13±0.46	37.93±0.33*	37.48±0.38***	37.33±0.23***	37.16±0.41***
IA	10	38.06±0.19	38.63±0.61	38.60±0.17	38.55±0.42	38.33±0.73	38.21±0.25
	100	38.00±0.19	38.60±0.29	38.58±0.16	38.41±0.55	38.26±0.37	38.15±0.48
IB	10	38.06±0.16	38.58±0.55	38.38±0.37	38.16±0.48	38.15±0.37	38.13±0.37
	100	38.08±0.19	38.55±0.32	38.18±0.19	38.16±0.66	38.15±0.60	38.03±0.32
IC	10	38.03±0.22	38.58±0.29	38.53±0.30	38.36±0.42	38.20±0.28	38.15±0.40
	100	38.06±0.19	38.63±0.29	38.35±0.24	38.21±0.44	38.01±0.29	38 .00±0.48
ID	10	38.08±0.19	38.54±0.38	38.10±0.21	37.98±0.73*	37.83±0.28*	37.68±0.53*
	100	38.06±0.19	38.50±0.48	38.05±0.50	37.65±0.52**	37.42±0.37***	37.38±0.48**
IE	10	38.00±0.10	38.65±0.26	38.55±0.30	38.51±0.44	38.43±0.36	38.36±0.19
	100	38.03±0.13	38.61±0.27	38.50±0.14	38.45±0.45	38.36±0.39	38.33±0.52
IF	10	38.10±0.21	38.60±0.28	38.01±0.54	37.80±0.50*	37.65±0.34**	37.53±0.23*
	100	38.06±0.19	38.65±0.23	38.01±0.55	37.65±0.54**	37.45±0.36***	37.41±0.36**
IG	10	38.00±0.10	38.60±0.29	38.56±0.27	38.45±0.13	38.33±0.37	38.11±0.27
	100	38.03±0.19	38.61±0.27	38.45±0.15	38.25±0.49	38.16±0.15	38.05±0.47
IH	10	38.06±0.19	38.58±0.29	38.13±0.59	37.83±0.31*	37.67±0.20**	37.51±0.23*
	100	38.10±0.21	38.58±0.29	38.00±0.17	37.45±0.72***	37.41±0.50***	37.31±0.28**
II	10	38.06±0.19	38.58±0.29	38.30±0.31	38.26±0.55	38.03±0.39	38.01±0.33
	100	38.06±0.19	38.58±0.29	38.28±0.45	38.11±0.21	38.01±0.61	37.96±0.28
IJ	10	38.00±0.10	38.60±0.29	38.05±0.30	37.73±0.32**	37.70±0.42**	37.55±0.15*
	100	38.06±0.19	38.60±0.24	37.98±0.48	37.55±0.30***	37.36±0.27***	37.28±0.24***

Values are Mean ±SD, n=6 in each group.*P<0.05, **P<0.01, ***P<0.001 when compared with control group (Newman-Keuls multiple comparison test)

DISCUSSION

Isatin-3-[N²-(2-benzalaminothiazol-4-yl)] hydrazone derivatives (IA-IJ) were evaluated for anti-inflammatory, analgesic and antipyretic activity by using standard experimental models. In the present investigation carrageenan induced paw edema acute inflammatory model, isatin derivatives inhibited inflammation effectively by cyclooxygenase inhibition. Acute inflammation is belived to be biphasic, the first phase (1hr) involves the release of serotonin and histamine while the second phase (over 1 hr) is mediated by prostaglandins, the cyclooxygenase products and continuity between two phases is proved by kinins.[19-21] Among all the derivatives ID, IF, IH and IJ at 100 mg/kg dose showed significant paw edema inhibition and this activity of isatin derivatives is mainly due to substitution at 5th position comparable with previously published studies of different substitutions at 5th position of isatin.[22]

The evaluation of analgesic activity by Eddy's hot plate method, where the mechanical stimulations can evoke the pain by increasing the synthesis of pain mediators (PG's, histamine, kininsetc)[23] through pain receptors and reaction time is noted as therapeutic end point. From the Graph 1 and 2, among all ID, IF, IH and IJ at dose of 100 mg/kg showed increase in reaction time (analgesic activity).This suggested that compounds showed inhibitory actions on cyclooxygenase mediated pathway.

In Brewer's yeast induced pyrexia model, evaluation in the body temperature is due to the fever mediators such as IL-Ib ,IL-6, IFN-α and prostaglandins (PGE_2) especially in the brain regions. The indole derivatives showed bradykinin and peptide synthesis inhibition.[24] As compared with other derivatives the ID, IF, IH and IJ were shown reduction of body temperature at 100 mg/kg when compared with control. The mechanism in reduction of fever is probably by fever mediator's synthesis inhibition.

CONCLUSION

Thus, we concluded that novel isatin derivatives produce significant anti-inflammatory , analgesic and antipyretic activities and this is suggesting that these derivatives has profound inhibitory actions on cyclooxygenase enzymes .But it should be suggested that further exact mechanism of action is necessary.

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