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Synthesis Of Some Mannich Bases Of Isatin Derivatives For Their Possible Biological Activities

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ABSTRACT: Isatin (1H-indole-2,3-dione) has been reacted with substituted anilines to form Schiff bases and the corresponding N-Mannich bases of these compounds were synthesized by reacting with formaldehyde and secondary amine. The chemical structures of the title compounds have been confirmed and elucidated by means of their physical and spectral data respectively. The compounds were tested for their possible antibacterial, analgesic and anti-inflammatory activities by the standard methods. Among the tested compounds, the compound containing chloro group showed the most favorable activity.

Key words: Isatin, Mannich bases, Antibacterial, Analgesic, Anti-inflammatory.

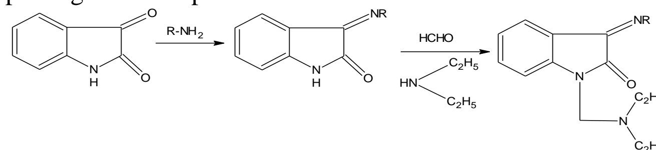
INTRODUCTION Schiff and Mannich bases of isatin represent one of the important classes of organic compounds because of their broad spectrum of pharmacological activities such as antibacterial[1-4], antifungal[5-6], antiviral[7-8], anti-HIV[9-12], antidepressant[13], anticonvulsant[14-16], analgesic[17] and anti-inflammatory[18] activities. The good biological profile of isatin derivatives prompted us to synthesize some Mannich bases of isatin. In the present study, aromatic primary amines were subjected to reaction with isatin to form Schiff bases. The corresponding N-Mannich bases were synthesized by reacting them with the secondary amine and formaldehyde. The chemical structures of the synthesized compounds were confirmed by means of their physical, IR, ^{1}H-NMR and Mass spectral data. The synthesized compounds were tested for their antibacterial activity by cup plate method, analgesic activity by acetic acid induced writhing response, hot plate reaction time and tail immersion method and anti-inflammatory activity by carrageenan induced paw edema method.

EXPERIMENTAL

The melting points were determined in open capillaries by using a Thomas Hoover melting point apparatus, expressed in ^{o}C and are uncorrected. The IR spectra of the compounds were recorded on Shimadzu IR Affinity series-1 in KBr and the values are expressed in cm^{-1}. The ^{1}H-NMR spectra of the compounds were recorded on a Bruker Advance II 400 MHz spectrophotometer and the values were expressed in δ ppm. The mass spectra of the compounds were recorded on Micromass Q-Tof Micro; in *m/z*. The purity of the compounds was checked by thin layer chromatography on silica gel G coated plates.

Synthesis of Schiff bases of isatin: Equimolar (0.01 mol) quantity of isatin and substituted anilines were dissolved in a sufficient amount of ethanol and refluxed for 3 hr in presence glacial acetic acid. After standing for approximately 24 hr at room temperature, the products were separated by filtration, dried under vacuum and recrystallized from warm ethanol.

Synthesis of Mannich bases of isatin: Equimolar quantity of diethylamine (0.004mol) in 10ml of ethanol was added to slurry containing appropriate isatin and formaldehyde solution (37% v/v) in 10 ml of ethanol. The reaction mixture was stirred for 2 hr at room temperature and kept under refrigeration for 48 hr. The products were separated by suction filtration, dried under vacuum and recrystallized from ethanol. The molecular formula, molecular weight, melting point, yield, R_f and spectral data were presented in table-1 and 2. TLC was monitored by using solvent system benzene: chloroform (55:45) and the spots were identified by placing the dried plate in iodine chamber.



Antibacterial study: The synthesized compounds were screened *in-vitro* for their antibacterial activity against *Staphylococcus aureus* (MTCC-87), *Escherichia coli* (MTCC-40), *Staphylococcus epidermidis* (MTCC-2639), *Pseudomonas aeruginosa* (MTCC-424) and *Proteus vulgaris* (MTCC-426) using cup plate method[19]. The

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compounds were tested at 500µg concentration in DMSO, using nutrient agar as the medium. After 24hr of incubation at 37°C, the zone of inhibition formed were measured in mm against standard drug tetracycline and the data were presented in table-3.

Analgesic activity: The analgesic activity of the synthesized compounds was studied using acetic acid induced writhing response[20], hot plate reaction time[21] and tail immersion method. In the three models, acetic acid induced writhing response (chemical method), hot plate reaction time and tail immersion method (thermal method), the animals were divided into different groups (n=6). Group I served as control (1% Carboxy Methyl Cellulose as vehicle, 1ml/kg, p.o.), group II served as standard (Indomethacin, 10 mg/kg, p.o. in Acetic acid induced writhing method and Pentazocine, 10 mg/kg, p.o. in Hot plate and Tail immersion methods) and other groups were served as test groups and received the test compounds each at the dose of 200 mg/kg/p.o. The vehicle, standard and test compounds were administered in the suspension form in Carboxy Methyl Cellulose to the respective groups, 30 min before the induction of pain, either by acetic acid or by thermal stimuli.

Acetic acid induced writhing method: Healthy Swiss albino mice (20-30 gm) were placed into individual restraining cages. The animals were then allowed to adapt in the cages for 30 minutes before testing. Writhing was induced in mice by administration of 0.6% acetic acid (10 ml/kg body weight, i.p.). The number of writhes was calculated over the period of 20 min after acetic acid injection. A writh is indicated by an abdominal constriction followed by full extension of hind limb. The data represent the total numbers of writhes observed over the 20 min period.

Hot plate method: The hot plate test in mice was performed by using Eddy's hot plate (INCO) maintained at a temperature of 55±1°C. The animals which showed fore paw licking or jumping response within 6-8 sec were selected for the study. The animals were individually exposed to the hot plate maintained at 55 ± 1°C. A cut off period of 15s was observed to avoid damage to the paw[22]. The time taken in sec for fore or hind paw licking or jumping was taken as reaction time.

Tail immersion method: Healthy Wistar rats (150-200 gm) were placed into individual restraining cages leaving the tail hanging out freely. The animals were then allowed to adapt in the cages for 30 minutes before testing. The lower 5 cm portion of the tail was marked and immersed in a cup of freshly filled water of 55°C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded by a stop watch. After each attempt the tail was carefully dried. The reaction was determined before oral feeding of the drug and the test compounds which was recorded as zero minutes reading. After the drug administration the reaction time was recorded at 1st hour and 2nd hour. The mean reaction time was recorded for each group and compared with the control.

Anti-inflammatory study: The anti-inflammatory activity was determined by carrageenan induced paw edema method[23] in Wister rats by using digital plethysmometer (Panlab LE 7500). Wister rats of either sex (180-250 gm) were selected and housed under standard laboratory conditions, given standard rat pellet and tap water ad libitum and maintained under standard environmental conditions throughout the period of experimentation. The animals were housed in cages under standard laboratory condition. They had free access to standard diet and water. The animals were divided into different groups of six animals each and fasted for 12hr before the experiment. Group I served as control and received vehicle, group II served as standard group and received indomethacin (10 mg/kg, p.o.) and other groups are served as test groups and received the test compounds (200 mg/kg/p.o.) one hour prior to carrageenan injection. The initial right hind paw volume of the rats were measured using a digital plethysmometer and then 0.1 ml of 1% w/v carrageen solution in normal saline was injected into the sub plantar region of the right hind paw. The volume of right hind paw was measured at 1, 2, 3, 4 and 5 hr after carrageenan injection by using digital plethysmometer. The data were expressed is paw volume (ml), compared with the initial hind paw volume of each rat.

Table-1: Physical data of the synthesized compounds

Compound	R	M.F.	M.W.	M.P. (^{o}C)	Yield (%)	R_f
8A	phenyl	$C_{19}H_{21}N_3O$	307.39	165-166	65.21	0.537
8B	2-nitrophenyl	$C_{19}H_{20}N_4O_3$	352.38	172-173	72.11	0.610
8C	3-	$C_{19}H_{20}$	352	17	75.	0.4

	nitrophenyl	N_4O_3	.38	0-171	15	69
8D	4-nitrophenyl	$C_{19}H_{20}N_4O_3$	352.38	165-166	69.66	0.663
8E	3-chlorophenyl	$C_{19}H_{20}ClN_3O$	341.83	157-158	77.11	0.656
8F	4-chlorophenyl	$C_{19}H_{20}ClN_3O$	341.83	155-156	81.20	0.678
8G	4-bromophenyl	$C_{19}H_{20}BrN_3O$	386.28	167-168	79.33	0.723
8H	4-fluorophenyl	$C_{19}H_{20}FN_3O$	325.38	150-151	82.32	0.620
8I	3-chloro-4-fluorophenyl	$C_{19}H_{19}ClFN_3O$	359.82	175-176	75.31	0.722
8J	2,6-dichlorophenyl	$C_{19}H_{19}Cl_2N_3O$	376.27	180-181	80.26	0.743
8K	2,4-dinitrophenyl	$C_{19}H_{19}N_5O_5$	397.38	168-169	83.61	0.605
8L	3,4-dichlorophenyl	$C_{19}H_{19}Cl_2N_3O$	376.28	176-177	84.92	0.682
8M	4-chloro-2-nitrophenyl	$C_{19}H_{19}ClN_4O_3$	386.83	173-174	66.78	0.582
8N	2-chloro-4-nitrophenyl	$C_{19}H_{19}ClN_4O_3$	386.83	176-177	76.68	0.552

Table-2: IR, ^{1}H-NMR and Mass spectral data of the synthesized compounds

Compd	**IR (KBr), ^{1}HNMR (400 MHz, DMSO-d_6), Mass (m/z)**
8A	IR (cm^{-1}): 1728(C=O), 1604(C=N), 1467(C=C) ^{1}H NMR δ (ppm): 1.05-1.13 (t, 6H, $N(CH_2CH_3)_2$), 2.61-2.72 (q, 4H, $N(CH_2CH_3)_2$), 4.42(s, 2H, CH_2), 7.06-7.67(m, 9H, Ar-H)
8B	IR (cm^{-1}): 1726(C=O), 1600(C=N), 1467(C=C),1529 &1354(NO_2) ^{1}H NMR δ (ppm): 1.01-1.05 (t, 6H, $N(CH_2CH_3)_2$), 2.51-2.66 (q, 4H, $N(CH_2CH_3)_2$), 4.49(s, 2H, CH_2), 6.42-8.07(m, 8H, Ar-H). *m/z* 353
8C	IR (cm^{-1}):1726(C=O), 1600(C=N),1467(C=C),1529 &1354(NO_2) ^{1}H NMR δ (ppm): 1.01-1.05 (t, 6H, $N(CH_2CH_3)_2$), 2.51-2.66 (q, 4H, $N(CH_2CH_3)_2$), 4.49(s, 2H, CH_2), 6.42-8.07(m, 8H, Ar-H). *m/z* 352
8D	IR (cm^{-1}): 1728(C=O), 1602(C=N), 1462(C=C), 759(C-Cl) ^{1}H NMR δ (ppm): 0.97-1.15(t, 6H, $N(CH_2CH_3)_2$), 2.50-2.65(q, 4H, $N(CH_2CH_3)_2$), 4.48(s, 2H, CH_2), 6.56-7.69(m, 8H, Ar-H). *m/z* 354
8E	IR (cm^{-1}): 1728(C=O), 1602(C=N), 1462(C=C), 759(C-Cl) ^{1}H NMR δ (ppm): 0.97-1.15(t, 6H, $N(CH_2CH_3)_2$), 2.50-2.65(q, 4H, $N(CH_2CH_3)_2$), 4.48(s, 2H, CH_2), 6.56-7.69(m, 8H, Ar-H)
8F	IR (cm^{-1}): 1728(C=O), 1602(C=N), 1462(C=C), 759(C-Cl) ^{1}H NMR δ (ppm): 0.97-1.15(t, 6H, $N(CH_2CH_3)_2$), 2.50-2.65(q, 4H, $N(CH_2CH_3)_2$), 4.48(s, 2H, CH_2), 6.56-7.69(m, 8H, Ar-H)
8G	IR (cm^{-1}): 1728(C=O), 1602(C=N), 1462(C=C), 759(C-Cl) ^{1}H NMR δ (ppm): 0.97-1.15(t, 6H, $N(CH_2CH_3)_2$), 2.50-2.65(q, 4H, $N(CH_2CH_3)_2$), 4.48(s, 2H, CH_2), 6.56-7.69(m, 8H, Ar-H)
8H	IR(cm^{-1}): 1716(C=O), 1612(C=N), 1444 (C=C), 1097(C-F) ^{1}H NMR δ (ppm): 1.31(t, 6H, $N(CH_2CH_3)_2$), 2.57(q, 4H, $N(CH_2CH_3)_2$), 3.28(s, 2H, CH_2), 6.49-7.91(m, 8H, Ar-H). *m/z* 326
8I	IR (cm^{-1}): 1728(C=O), 1602(C=N), 1462(C=C), 759(C-Cl) ^{1}H NMR δ (ppm): 0.97-1.15(t, 6H, $N(CH_2CH_3)_2$), 2.50-2.65(q, 4H, $N(CH_2CH_3)_2$), 4.48(s, 2H, CH_2), 6.56-7.69(m, 8H, Ar-H)
8J	IR (cm^{-1}): 1728(C=O), 1602(C=N), 1462(C=C), 759(C-Cl) ^{1}H NMR δ (ppm): 0.97-1.15(t, 6H, $N(CH_2CH_3)_2$), 2.50-2.65(q, 4H, $N(CH_2CH_3)_2$), 4.48(s, 2H, CH_2), 6.56-7.69(m, 8H, Ar-H)
8K	IR(cm^{-1}): 1753(C=O),1631(C=N), 1464 (C=C) ,1334 &1546 (NO_2)

	^{1}H NMR δ (ppm): 1.16-1.36(t, 6H, N($CH_2CH_3)_2$), 2.57-2.59(q, 4H, N($CH_2CH_3)_2$), 3.30-3.32(s, 2H, CH_2), 6.94-8.29(m, 7H, Ar-H)
8L	IR (cm^{-1}): 1716(C=O), 1614(C=N), 721(C-Cl), 1462(C=C) ^{1}H NMR δ (ppm): 1.25(t, 6H, ($CH_3)_2$), 2.57-2.58(q, 4H, N($CH_2CH_3)_2$), 4.47(s, 2H, CH_2), 6.55-7.80(m, 7H, Ar-H)
8 M	IR (cm^{-1}): 1728(C=O), 1602(C=N), 1462(C=C), 759(C-Cl) ^{1}H NMR δ (ppm): 0.97-1.15(t, 6H, N($CH_2CH_3)_2$), 2.50-2.65(q, 4H, N($CH_2CH_3)_2$), 4.48(s, 2H, CH_2), 6.56-7.69(m, 8H, Ar-H). *m/z* 389
8 N	IR (cm^{-1}): 1728(C=O), 1602(C=N), 1462(C=C), 759(C-Cl). ^{1}H NMR δ (ppm): 0.97-1.15(t, 6H, N($CH_2CH_3)_2$), 2.50-2.65(q, 4H, N($CH_2CH_3)_2$), 4.48(s, 2H, CH_2), 6.56-7.69(m, 8H, Ar-H)

Table-3. *In-vitro* Antibacterial activity of the synthesized compounds

Compounds	Diameter of zone of inhibition (mm)				
	P. vulgaris	*P. aeruginosa*	*E. coli*	*S. aureus*	*S. epidermidis*
8A	13.00 ± 1.00	-	11.33 ± 0.57	13.00 ± 1.00	12.33 ± 0.57
8B	14.00 ± 1.00	-	09.66 ± 0.57	12.33 ± 0.57	11.66 ± 0.57
8C	14.33 ± 0.57	14.33 ± 0.57	13.33 ± 0.57	16.33 ± 0.57	15.33 ± 0.57
8D	10.66 ± 0.57	12.00 ± 1.00	12.33 ± 0.57	14.33 ± 0.57	13.00 ± 1.00
8E	11.66 ± 0.57	10.33 ± 0.57	13.33 ± 0.57	12.33 ± 0.57	10.66 ± 1.52
8F	15.33 ± 0.57	-	12.66 ± 0.57	09.33 ± 0.57	09.66 ± 0.57
8G	12.33 ± 0.57	09.00 ± 1.00	09.66 ± 0.57	09.33 ± 0.57	07.33 ± 0.57
8H	11.00 ± 1.00	09.00 ± 1.00	13.66 ± 0.57	07.33 ± 0.57	09.66 ± 1.15
8I	10.66 ± 0.57	06.66 ± 0.57	06.66 ± 0.57	07.66 ± 0.57	06.33 ± 0.57
8J	21.00 ± 1.00	18.00 ± 1.00	26.00 ± 1.00	18.00 ± 1.00	20.66 ± 0.57
8K	16.33 ± 0.57	12.33 ± 0.57	26.66 ± 1.52	14.66 ± 0.57	17.33 ± 0.57
8L	12.33 ± 0.57	09.66 ± 0.57	10.33 ± 0.57	09.00 ± 1.00	09.33 ± 0.57
8M	20.66 ± 0.57	14.00 ± 1.00	27.33 ± 1.52	17.33 ± 0.57	16.33 ± 0.57
8N	16.33 ± 0.57	08.33 ± 0.57	14.33 ± 0.57	15.00 ± 0.00	15.00 ± 1.00
Control	-	-	-	-	-
Standard	23.33 ± 0.57	20.00 ± 1.00	31.66 ± 0.57	21.33 ± 0.57	23.66 ± 0.57

Results were expressed as Mean ± S.D. (n = 3), "-" indicates no zone of inhibition.

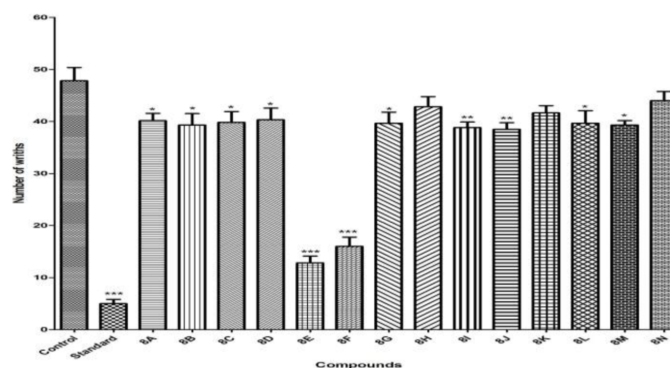

Fig.1. Effects of the synthesized compounds on acetic acid induced writhing in mice. Results were expressed as mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to control.

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Results were expressed as Mean ± SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001 as compared to control.

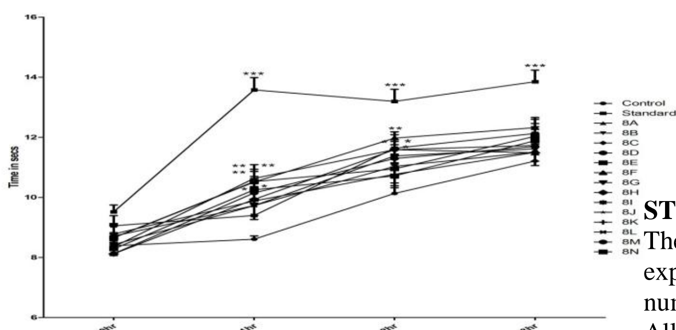

Fig.2. Effects of the synthesized compounds on latency time of mice exposed to hot plate test. Results were expressed as mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to control.

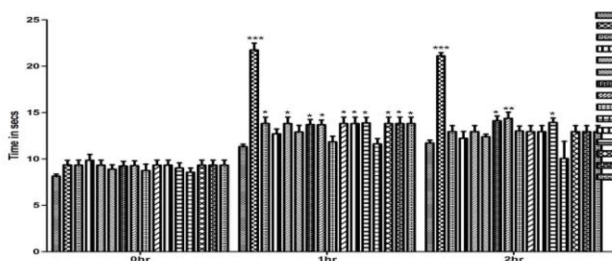

Fig.3. Effects of the synthesized compounds on latency time of mice exposed to tail immersion test. Results were expressed as mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to control

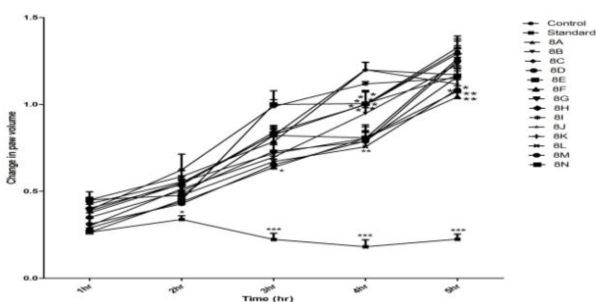

Fig.4. Effects of the synthesized compounds on the rat paw edema-induced by subplantar injection of carrageenan.

STATISTICAL ANALYSIS

The results of statistical analysis for animal experiments were expressed as mean ± SEM. The number of animals in each group were six (n=6). All the results were statistically analyzed by two way ANOVA followed by Bonferroni post tests except that of acetic acid induced writhing model where the data were analyzed by one way ANOVA followed by Dunnet's multiple comparison test by using GraphPad Prism software, v 5.0 (trial), (GraphPad Inc, USA). *p < 0.05, **p < 0.01, ***p < 0.001 compared to control were considered to be statistically significant.

RESULTS AND DISCUSSION

The spectral and physical data proved the structure and purity of the synthesized compounds. The synthesized compounds were evaluated for *in-vitro* antibacterial activity by cup plate method. The results were summarized in table-3 including the activity of standard. The compound 8J exhibited highest activity against *P. vulgaris. P. aeruginosa, S. aureus* and *S. epidermidis,* whereas the compound 8M showed highest activity against *E. coli*. But the activity was less than the standard drug Tetracycline in this test concentration. The analgesic activity of the synthesized compounds was evaluated using both chemical and thermal methods. Acetic acid induced writhing test used for detecting both central and peripheral analgesia, whereas hot plate and tail immersion methods are considered to be selective for the drugs acting centrally. Thermal methods are more sensitive to opioid μ receptors and non- thermal tests are to opioid k receptors. Intraperitoneal administration of acetic acid releases prostaglandins and mediators like PGE_2 and $PGF_{2\alpha}$ and their levels were increased in the peritoneal fluid of the acetic acid induced mice. Most of the tested compounds showed significant activity.

The compound 8A, 8B, 8C, 8D, 8G, 8L and 8M were significant (p< 0.05) in reducing the number of wriths whereas the compounds 8I and 8J were found to be significant (p < 0.01) in reducing the number of wriths, but the activity was comparatively less than that of 8E and 8F (p<0.001). The standard drug Indomethacin was found to be more potent than the test compounds (Fig.1). With respect to hot plate test the

compounds 8D and 8M ($p<0.05$) as well as 8E ($p < 0.01$) were found to be significant only in first hour whereas 8A and 8C showed significant activity ($p < 0.05$) only in the second hour. The compound 8F showed significant activity ($p < 0.01$) both in first and second hour. Similarly the compound 8J was found to be significant in first ($p < 0.01$) and second ($p < 0.05$) hour of the study. None of the compounds were active in third hour of the study except the standard drug Pentazocine (Fig.2). In tail immersion test the compounds 8A, 8C, 8E, 8I, 8J, 8L, 8M and 8N showed significant ($P < 0.05$) increase in latency in the first hour whereas the compounds 8E, 8F and 8I were found to have significant increase in latency both in first and second hour of the study (Fig.3). Carrageenan induced paw edema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic, the first phase (1hr) involves the release of serotonin and histamine while the second phase (over 1hr) is mediated by prostaglandins, the cyclooxygenase products and the continuity between the two phases is provided by kinins. The result of this study shows that some of the synthesized compounds have significant anti-inflammatory activity. Among these, the compound 8F showed significant anti-inflammatory activity at third ($p < 0.05$), fourth ($p < 0.01$) and fifth ($p < 0.01$) hour whereas the compound 8E was found to be significant at fourth ($p < 0.05$) and fifth ($p < 0.01$) hour. Similarly the compound 8L was also found to be significant at fourth ($p < 0.01$) and fifth ($p < 0.05$) hour. Again the compound 8J was found to be significant ($p < 0.05$) at fourth and fifth hour. The compounds 8A, 8C, 8D, 8G and 8M were significant ($p < 0.05$) only at the fourth hour of the study. Although some of the synthesized compounds showed significant anti-inflammatory activity, but they were not remarkably comparable to that obtained by the standard drug Indomethacin (Fig.4).

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