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Letter to the Editor

Anti-inflammatory activity of novel Schiff bases by *in vitro* models

Sir,

A comprehensive collection of enzyme activation, moderator release, cell relocation, tissue collapse and repair are involved in an inflammation reaction (Vane, 2000) for host defense and usually activated in most disease conditions. Currently research for new drugs or compounds which posses anti-inflammatory activity is going on which may lead to the discovery of new therapeutic agent which could be used to suppress the inflammation and in various disease conditions where the inflammation response in increase the disease process. An exhaustive investigation on different thiadiazole compounds have been done in recent years, many of which were found to posses wide spectrum of pharmacological actions. Moreover, various reports indicate that semicarbazides and thiadiazoles derivatives show anti-inflammatory (Labanauskas et al., 2001, Palaska et al., 2002) and analgesic (Amir and Shikha, 2004) activities. The present study was carried out to look into the anti-inflammatory activity of novel thiadiazole derivatives (**7a-f**) by different *in vitro* models for the first time.

Test compounds were synthesized in laboratory and compounds were checked for purity by TLC, IR, ^{1}H NMR and elemental analysis. Test compounds are as follows:

5-(2-Hydroxyphenyl)-2-{N-(4-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole (**7a**), 5-(2-Hydroxyphenyl)-2-{N-(4-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole (**7b**), 5-(2-Hydroxyphenyl)-2-{N-(4-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole (**7c**), 5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole (**7d**), 5-(2-Hydroxyphenyl)-2-{N-(4-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole (**7e**), 5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene) -4-aminophenyl}-1,3,4-thiadiazole (**7f**). All the chemicals used were purchased from Ideal Chemicals, Raipur. All the chemicals were of Merck and Lobachem. The Instrument used was of Model UV-1800, Shimadzu, Japan and cuvette used was of 1 cm path length and made up of quartz.

First model is the inhibition of protein denaturation which was evaluated by the methods with slight modification (Mizushima and Kobayashi, 1968; Sakat et al., 2010) with slight modification. 500 µL volume of 1% bovine serum albumin was added to 100 µL of test dilution. The mixture was kept at room temperature for 10 min, then heated for 15-20 min at 51°C. Then the solution was brought to normal room temperature and absorbance at 660 nm was recorded. Acetyl salicylic acid was used as standard. The process was carried out in triplicates and protein denaturation inhibition percentage was calculated using formula:

$$\% \text{ Inhibition} = 100 - [(A_1-A_2) / A_0] \times 100$$

where, A_1 is sample absorbance, A_2 is product control absorbance and A_0 is the absorbance of the positive control

Second model is RBC membrane stabilization or Inhibition of RBC hemolysis. The blood was collected from healthy human volunteer, who had not taken any NSAIDS for last 2 weeks earlier to the experiment and mixed with equal volume of Alsever's solution (2% dextrose, 0.7% sodium citrate, 0.5% citric acid and 0.4% NaCl). The mixture was centrifuged for 10 min at 3,000 rpm. The obtained solution was washed with saline three times. RBC layer was collected and diluted with phosphate buffer saline (PBS) to make 10% v/v solution (Sadique et al., 1989, Saket et al., 2010). 100 µL of 10% RBC solution was added to 100 µL of test dilution. The resulting solution was heated at 56°C for 30 min followed by centrifugation at 2,000 rpm for 8-10 min at room temperature. Clear supernatant was collected, and absorbance was recorded at 560 nm. Membrane stabilization percentage was calculated by the method as described elsewhere (Shinde et al., 1999, Sakat et al., 2010).

Third model is proteinase inhibition assay. Trypsin inhibition was evaluated by the method described elsewhere (Oyedepo and Femurewas, 1965; Sakat et al., 2010). 100 µL of 1% bovine serum albumin was added to 100 µl of test dilution. The mixture was incubated at room temperature for 5-6 min. Reaction was inhibited by adding 250 µL of trypsin followed by centrifugation. The supernatant was separated and absorbance was recorded at 210 nm. Acetylsalicylic acid was used as standard and proteinase inhibition percentage was calculated

Inhibition of albumin denaturation by test compounds was studied. Selected test compounds were effective in

Table I

Anti-inflammatory activity of novel Schiff bases by *in vitro* models

Concentration (µg/mL)	*In vitro* models	% Inhibition					
		7a	7b	7c	7d	7e	7f
50	Albumin denaturation	23.4 ± 1.0	24.8 ± 0.4	25.8 ± 0.6	25.9 ± 0.7	28.1 ± 0.3	29.8 ± 0.4
	Membrane stabilization	17.8 ± 0.6	17.3 ± 0.6	21.6 ± 0.4	29.1 ± 0.7	32.3 ± 1.3	33.5 ± 1.1
	Proteinase inhibition	17.4 ± 0.5	19.5 ± 0.4	20.9 ± 0.9	21.5 ± 0.4	23.8 ± 0.8	25.9 ± 0.6
100	Albumin denaturation	38.9 ± 0.5	38.6 ± 0.3	40.6 ± 0.7	40.9 ± 0.3	42.9 ± 0.1	45.4 ± 1.0
	Membrane stabilization	22.3 ± 0.7	29.8 ± 0.4	33.7 ± 0.5	37.1 ± 0.7	42.6 ± 0.7	45.0 ± 1.1
	Proteinase inhibition	33.5 ± 0.7	32.8 ± 0.8	33.9 ± 0.2	34.7 ± 0.6	38.0 ± 0.2	40.9 ± 0.4
200	Albumin denaturation	47.8 ± 0.3	49.9 ± 0.3	51.7 ± 0.3	50.9 ± 0.3	53.7 ± 0.6	58.1 ± 0.5
	Membrane stabilization	38.2 ± 0.9	32.6 ± 0.5	42.8 ± 0.9	44.3 ± 0.6	47.8 ± 0.8	53.2 ± 0.3
	Proteinase inhibition	36.2 ± 0.4	38.6 ± 0.8	41.5 ± 0.6	40.6 ± 0.4	47.9 ± 0.8	50.8 ± 0.6

Results are expressed as Mean ± SEM

inhibiting heat induced albumin denaturation. IC_{50} of most potent **7f** was observed as 147.6 µg/mL. Results are shown in Table I. Acetylsalicylic acid was used as standard anti-inflammation drug. Inhibition of RBC hemolysis or RBC membrane stabilization effect by test compounds was studied. The compounds inhibited the heat induced hemolysis of RBCs. IC_{50} of **7f** was observed as 167.1 µg/mL. Proteinase inhibition activity of test compounds was studied. The test compounds exhibited significant anti-proteinase activity. Maximum inhibition was observed in **7f** and IC_{50} were observed as 186.3 µg/mL.

The main cause of inflammation is denaturation of protein. Anti-inflammatory drugs like phenybutazone have been found to posses ability to thermally induce protein denaturation (Mizushima and Kobayashi, 1968). The ability of the test compound to inhibit protein denaturation was studied as a part of study on the mechanism of the anti-inflammatory activity. The test compounds showed relationship as increase in % inhibition with increase in concentration. To further prove the mechanism of anti-inflammatory action of test compounds, Stabilization of RBC membrane was studied. All compounds effectively inhibited the heat induced hemolysis. The results provide evidences for membrane stabilization effect of the test compounds as an additional mechanism for their anti-inflammatory effect. Due to the resemblance of RBC membrane with lysosomal membrane, this effect may possibly inhibit the release of neutrophils' lysosomal content at the location of inflammation. The compounds suppressed the RBC haemolysis. Even though specific mechanism of membrane stabilization is yet to be known, it may be possible that the test compounds show the effect by maintaining the surface area/volume ratio of the cell. This could be done by growth of membrane and or contraction of the cell and interaction with membrane proteins (Shinde et al., 1999). Proteinase has been implicated in arthritics. Neutrophils are a wealthy source of proteinase which carries many serine proteinases in their lysosomal granules. Formerly it was reported that proteinase of leukocytes play an important role the formation of tissue break in inflammation reactions and considerable level of protection was brought by proteinase inhibitors (Das and Chatterjee, 1995). The test compounds exhibited significant antiproteinase activity.

Ajit Kumar Pandey, Pranita P. Kashyap and Chanchal Deep Kaur

Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh 490042, India.

Corresponding author:
email: ajitpandey588@gmail.com

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