Evaluation of antipyretic potential of new heterocyclic derivatives of 3-formyl-4-hydroxycoumarin in rats

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Introduction
Inflammation is a body defense reaction to eliminate or limit the spread of an injurious agent and is characterized by five cardinal signs, redness, swelling, heat, pain and loss of function. The inflammatory process involves a cascade of events elicited by numerous stimuli that includes infectious agent, ischemia, antigen-antibody interaction and thermal or physical injury. Non-steroidal anti-inflammatory drugs are widely used in the treatment of acute and chronic inflammation, pain and fever. But the greatest disadvantage in the presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation. Therefore, there is a dire need for screening and development of novel, but better antipyretic drugs. Coumarins have multiple biological activities; various coumarin-related derivatives are recognized as inhibitors of the lipooxygenase and cyclooxygenase pathways of arachidonate metabolism. Several natural or synthetic coumarins with various hydroxyl and other substitutes were found to inhibit lipid peroxidation and to scavenge hydroxyl radical and superoxide anion and to influence processes involving free radical mediated injury. The objective of the present study was to evaluate the antipyretic activity of various heterocyclic derivatives of 3-formyl-4-hydroxycoumarin (synthesized by us) in animal models. All compounds synthesized were evaluated for the above activity and their effects were compared with the standard drugs. In our study, a new model of pyrexia suggested by Tomazetti et al., 2005 was used where backer’s yeast is used to induced pyrexia in juvenile male Wistar rats, the rats received Backer’s yeast at 135mg/kg intraperitonealy. The test compound or Paracetamol was given orally 1 hour after injection of yeast (when average rise in rectal temp was about 1°C). The test compounds (heterocyclic derivatives of 3-formyl-4-hydroxycoumarin) showed significant antipyretic activity in mice.

Materials and methods
This interdepartmental study was conducted in collaboration of department of chemistry AMU, Aligarh and department of pharmacology, JNMC, AMU, Aligarh.

2.1 Chemicals and test compounds:
The following heterocyclic compounds were synthesized in the research laboratory of Department of Chemistry and studied for their physicochemical and spectral properties (Siddiqui and Asad, 2006). They were tested for antipyretic activity in animal models.

1. 3-Acetocacetyl pyrano [3, 2-c][1] benzopyran 2, 5-dione (fig.1) was prepared from intramolecular transactonization of 4-hydroxycoumarins and triacetic acid lactone. The resulting compounds 1, which possessed a 1, 3-diketone unit in its structure were converted to pyrazoles by treatment with hydrazine, phenylhydrazine and hydrazinobenzothiazole to afford

1a. 3-(3-methyl pyrazol-5-yl)-pyrano [3, 2-c][1] benzopyran 2, 5-dione.
1b. 3-(3- methyl-1-phenyl pyrazol-5-yl) - pyrano [3, 2-c] benzopyran-2, 5-dione and
1c. 3-(3-methyl-1-benzothiazolopyrazol-5-yl)-pyrano [3, 2-c][1] benzopyran-2, 5-dione.

The test compounds were dissolved in 2.5% DMSO (Dimethyl sulphoxide) prior to administration in different concentration so that animal received equal volume each time (5 mI/kg). The dose selection of the test compound was based on preliminary trial carried out in our laboratory over a dose range 5 mg/kg to 40 mg/kg in geometric increasing order and maximal effect was found at the dose of 20 mg/kg.

Drugs used: Baker’s yeast (Britannia food products) and Paracetamol (IPCA).

2.2. Experimental Animals: Young male Wistar Albino rats 28-
30 days of age (weight 90-100 g) (Tomazetti et al., 2005) were used. The animals were obtained from the Laboratory Animal Breeding and Research Center Jamia Hamdard, New Delhi. The animals were given a week time to get acclimatized with laboratory conditions. The animals were housed in polypropylene cages (4 per cage) with sterilized paper cuttings as bedding material under laboratory conditions with control environment of temperature 22±3°C, humidity (60% ± 10%) and 12 h light/dark cycle. They were given free access to food with standard rodent pellet diet (from Lipton India) and drinking water. The animals were transferred to the experimental room 2 hours before the experiment. The temperature reading were recorded between 10:00 h to 16:00 h, when the rectal temperature reported to be stable (Yochim JM 1968). The study protocol was approved by the institutional ethical committee.

2.3. Experimental Protocol: The following experimental models were used for test compounds.

a. Baker's yeast induced pyrexia: To test the effects of test compounds and Paracetamol on Baker's yeast induced pyrexia, rats were divided into four groups (n=6), the rats were set in their cages individually throughout the experiment, rectal temperature was measured with a lubricated thermometer probe inserted 3 cm deep into the rectum. The probe was linked to telethermometer (range 31-41°C with 0.1°C precision). Rectal temperature was measured recorded manually at intervals of every 15 minutes for each 5 hour. To minimize the stress response of the animals to the lightly restrained condition, we made a careful handling and performed two sets of acclimating training in the cage for 2 days before starting the experiments. Fever was induced by intraperitoneal injection of baker's yeast 135mg/kg, which induced a sustained increase in rectal temperature for 5 hr. Paracetamol and other novel antipyretics, reverted baker yeast-induced fever (Tomazetti et al., 2005). The test compounds and Paracetamol was administered one hour after injecting yeast when there was an average increase in temp of about 1°C.

Group I: (Control) only yeast was injected and continuously temperature was monitored and recorded at specified interval for 5 hours.

Group II: DMSO was given orally one hour after administering yeast and continuously temperature was monitored and recorded at specified interval for 5 hours.

Group III: Test drug was administered orally 1 hr after administering yeast and continuously temperature was monitored and recorded at specified interval for 5 hours.

Group IV: Paracetamol (150mg/kg) was given orally 1 hr after administering yeast and continuously temperature was monitored and recorded at specified interval for 5 hours.

d. Effect of test compounds and Paracetamol on basal rectal temperature: Test compounds and Paracetamol was given orally and rectal temperature was measured every 15 min for 5 hour and recorded manually at specified intervals for each group.

Group I: Received 2.5% DMSO (0.5 ml) given orally.

Group II: Test drug (dissolved in 0.5 ml DMSO) was administered orally.

Group III: Paracetamol (150mg/kg) was given orally.

c. Toxicity study
The acute oral toxicity was carried out as per the guideline set by the organization for the economic co-operation and development (OECDB) received from the committee for the purpose of control and supervision of experimental animals (CPCEA).

Experimental design and drug treatment: (Bruce. R-D 1985: Fundam Appl. Toxicol 5: 151-157), 2 rats (one from either sex) were dosed at predetermined (250, 500 and 1000 mg/kg dissolved in fixed amount (1.5 ml of DMSO) and administered by stomach feeding cannula. They were observed continuously for the first 2 hr for toxic symptoms and up to 24 hr for mortality (Litchfield et al., 1949). If there was no mortality or if no more than one rat of either sex died at the highest level tested (1000 mg/kg) with the total of 10 rats (5/sex) dosed at 1000 mg/kg and monitored for 7 days period LD50 was considered to more than 1000 mg/kg.

f. Statistical analysis
All values are presented as mean ± S.E.M. of six rats and difference between means were assessed by one-way analysis of variance (ANOVA), followed by student’s t test. Difference between means were considered to be significant at P<0.05 as compare to control.

Results
3.1. a. Effect of test compounds on Yeast-induced hyperpyrexia
The experimental rats showed a mean increase of about 1°C in rectal temperature 1 h after Baker's yeast injection (135mg/kg, i.p). The test compound (I, Ia and Ib) produced significant (P<0.05) antipyretic activity at 1 and 2 h after drug administration, whereas test compound Ib and the reference drug Paracetamol (150 mg/kg) showed significant antipyretic activity throughout the observation period up to 5 hr (Fig 2).

3.1. b. Effect of test compounds and Paracetamol on basal rectal temperature
The results showed by the test compound and paracetamol on normal body temperature in rats is presented in fig.3. The test compound I caused significant lowering of body temperature up to 2 hr (0.5°C) following its administration. While the maximum lowering of the rectal temperature noticed with the test compound Ib was 0.2°C up to 1 hr to 3 hr and that of standard drug paracetamol was 0.1°C up to 1 to 2 hr period.

3.3. Acute Toxicity study evaluation
In acute toxicity study the test compounds did not show any toxicity and mortality up to maximum dose of 1000 mg/kg body weight in rats. No gross change in behavior was observed at this dose. Weight of rats had a normal variation after 7 days of observations.

Discussion & Conclusion
Various coumarin-related derivatives are recognized as inhibitors of lipoygenase and cyclooxygenase pathways of arachidonate metabolism (Neichi, et al., 1983) but also of neutrophile-dependent super oxide anion generation (Ozaki, et al., 1986). Several natural or synthetic Coumarins with various hydroxyl and other substituents were found to inhibit lipid peroxidation and to scavenge hydroxyl radicals and superoxide anion (Paya, et al, 1992) and to influence processes involving free radical-mediated injury, as can some plant phenolics and flavonoids.

The present study has demonstrated the pharmacological potential of the synthetic new heterocyclic derivatives of 3-formyl-4-hydroxycoumarin with addition of different groups as an antipyretic agent when tested on various animal models.

The test compounds (I, Ia and Ic) revealed weak antipyretic effect but Ib produced marked antipyretic activity in Backer's yeast induced febrile rats its effect is comparable to that of the standard antipyretic drug paracetamol. In general, non-steroidal antiinflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus (Clark WO et al: 1975 and Zeit R et al; 1975). Various coumarin-related derivatives are recognized as inhibitors of lipoygenase and cyclooxygenase pathways of arachidonate metabolism (Neichi, et al., 1983), though there is no direct evidence of coumarin to interfere with prostaglandin synthesis in hypothalamus. In general, several mechanisms of action could be used to explain the observed antipyretic activity of the test compounds. The ability to inhibit/reverse the centrally synthesized prostaglandins or COX (Uzcátegui B et al, 2004) could be one of the possible mechanisms.
that contribute to the antipyretic activity of the test compounds seen in the present study. To conclude the synthetic new heterocyclic derivatives of 3-formyl-4-hydroxy Coumarins have potent antipyretic activity. Additions of different functional groups have varying effects. Significant increase in antipyretic effect of compound I was observed after addition of phenylhydrazine.

Table 1: The Effect of test compounds and Paracetamol on yeast-induced pyrexia in rats

<table>
<thead>
<tr>
<th>Test Compounds</th>
<th>Dose/kg</th>
<th>Temperature in OC</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
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<td>DMSO 5ml</td>
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<td>32.70 ± 0.23</td>
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<td>1 20 mg</td>
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<td>32.58 ± 0.23</td>
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<tr>
<td>1a 20 mg</td>
<td></td>
<td>32.61 ± 0.25</td>
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<tr>
<td>1b 20 mg</td>
<td></td>
<td>32.61 ± 0.25</td>
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<tr>
<td>1c 20 mg</td>
<td></td>
<td>32.58 ± 0.23</td>
</tr>
<tr>
<td>Paracetamol 150 mg</td>
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*P value of < 0.05 was considered as significant in comparison to control

Table 2: The Effect of test compounds on basal rectal temperature of rats.

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Fig 1: Structure of some novel new heterocyclic derivatives of 3-formyl-4-hydroxycoumarines synthesized and screened

Fig 2: Effect of test compounds and Paracetamol on yeast-induced pyrexia in rats

Fig 3: Effect of test compounds on basal rectal temperature of rats

Reference


