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A Refined *Saccharomyces Cerevisiae*-Induced Pyrexia Model In Rats For Specific Antipyretic Preclinical Screening

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ABSTRACT

Introduction. A fever-specific, reliable animal model is necessary to screen the antipyretic activity of pharmacological agents, especially to differentiate their action from broad anti-inflammatory activity. The present investigation was directed towards standardizing the yeast-induced pyrexia model in Sprague Dawley rats for screening the antipyretic activity of paracetamol. **Methods.** Male Sprague Dawley rats were placed into three groups (5 each): normal control, pyrexia-induced untreated, and paracetamol-treated. Pyrexia was induced by subcutaneous injection of 40% aqueous suspension of *Saccharomyces cerevisiae* (10 mL/kg b.w.). Paracetamol-treated rats were given a single oral dose of 150 mg/kg following pyrexia induction. Rectal temperature was measured at intervals of 30 minutes for 180 minutes. **Results.** *Saccharomyces cerevisiae* injection elicited a satisfactory febrile response in both pyrexia-induced groups. In the paracetamol-treated group, there was a considerable decrease in rectal temperature from 90 minutes, and the difference was statistically significant ($p < 0.05$) when compared with the untreated group. The model was able to distinguish the antipyretic effect of paracetamol from natural thermoregulatory fall in controls. **Conclusion.** This S. cerevisiae pyrexia model in mice is a specific and reproducible platform for antipyretic drug evaluation. The ability to dissociate antipyretic mechanisms from accompanying inflammatory processes is what makes it an acceptable model for future pharmacological screens. The addition of fever-specific biomarkers, i.e., hypothalamic metabolites and PGE_2, is suggested to also offer mechanistic insight and translational value.

1. Introduction

Fever is characterized by an elevation in core body temperature resulting from an imbalance between heat production and heat loss mechanisms. This imbalance may arise due to exposure to pyrogenic stimuli or impairment of the body's thermoregulatory processes. The thermoregulatory center located in the hypothalamus is primarily responsible for maintaining thermal homeostasis and responding to pyrogen-induced signals.[1] Fever is a highly conserved physiological response across diverse animal taxa, serving as a critical adaptive mechanism against infection and inflammation.[1,2]

Saccharomyces cerevisiae is a eukaryotic yeast extensively utilized in the food and biotechnology industries, recognized for its adaptability to extreme environmental conditions, particularly through post-translational modifications.[3] Commonly known as baker's yeast or brewer's yeast, *S. cerevisiae* plays a crucial role in inducing fever through inflammatory and neural mechanisms. When introduced into the body via injection, the immune system responds by upregulating the production of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and prostaglandin E2 (PGE2), key mediators in the febrile response. These elevated cytokine levels contribute to fever or pyrexia, a process regulated by the central nervous system, which orchestrates immune, neural, and endocrine responses to maintain homeostasis.[1,4]

Yeast-induced pyrexia in rats is one of the most widely used animal models for studying fever due to its reproducibility and well-characterized inflammatory response. Experimental evidence indicates that yeast-induced fever leads to a

significant increase in pro-inflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which play central roles in mediating the febrile response.[5] These cytokines serve as reliable biomarkers for evaluating the efficacy of antipyretic agents. However, small rodents such as rats and mice exhibit thermoregulatory limitations, as their ability to sustain a pronounced febrile response is relatively low.[6]

The limited febrile response in small rodents presents challenges in antipyretic research, often necessitating the use of larger test animals, which increases drug dosage, costs, and logistical constraints. Enhancing the reliability and translational relevance of fever models in small rodents is crucial for improving feasibility in preclinical studies. Therefore, this study aimed to refine existing pyrogen-induced fever models to establish a method for antipyretic research.

2. Methods

Materials

Paracetamol; Sanmol (PT. Sanbe Farma, Indonesia), Brewer Yeast from *Saccharomyces cerevisiae* (YSC1 Sigma-aldrich), Rat Food (CitraFeed), aquadest, rectal thermometer (Onemed), spuit (Thero), Sonde, and Water for injection (Otsuka).

Animals

Sprague Dawley male rats, aged 8 weeks and weighing between 150-250 grams, were obtained from the Bogor Agricultural University. The animals were maintained under standard laboratory conditions (25°C with a 12-hour light/dark cycle). The subjects were acclimatized for two weeks, with food and water provided regularly each day (a fasting treatment was implemented one day prior to the experiment). The experimental protocol was approved by the Research Ethics Committee (REC) of the Faculty of Medicine, National Development University Veteran, East Java, Indonesia (42/V/2025/KEP).

Experimental Groups

The rats were randomly allocated into three experimental groups: a normal control group, a negative control group, and a paracetamol-treated group. Each group consisted of five male rats (n=5), for a total of 15 animals. The sample size was determined based on previously published pyrexia models[7], and ethical considerations guided the minimization of animal usage while ensuring scientific validity. Rats in the normal control group received no interventions (neither pyrogen administration nor paracetamol treatment). Rats in the negative control group received a subcutaneous (s.c.) injection of pyrogen but were not treated with paracetamol. Rats in the paracetamol-treated group received both a subcutaneous (s.c.) pyrogen injection and subsequent paracetamol 150 mg/kg treatment administered orally (p.o.). Dosages for pyrogen and paracetamol were individually calculated and adjusted based on the rats' respective body weights, ensuring standardized treatment across all groups.

Pyrogen Induction

Rats were administered a single subcutaneous (SC) injection of a 40% aqueous suspension of Brewer's yeast (*Saccharomyces cerevisiae*) at a 10 mL/kg body weight. The dose was separately calculated for each animal according to the animal's weight for appropriate administration. Rectal temperature was measured for a period of 180 minutes at intervals of 30 minutes, thus providing a total of seven time points to compare.

Thermal Homeostasis Observations

Rectal body temperature measurements were performed in rats following subcutaneous (s.c.) injection of *Saccharomyces cerevisiae* (yeast) to induce pyrexia, and subsequent administration of a single oral dose of paracetamol. Administration of pyrogen resulted in a significant elevation in rectal temperature (fever). Following oral dosing of paracetamol, a gradual decrease in rectal temperature was observed during the measurement period. Rectal temperatures were recorded at 30-minute intervals over a total duration of 180 minutes.

Statistical Analysis

The study used a randomized controlled design with three groups: normal control, pyrexia-induced untreated, and pyrexia-induced paracetamol-treated. Temperature changes were analyzed by repeated-measures ANOVA with post hoc tests. A p-value <0.05 indicated significance, enabling robust assessment of paracetamol's antipyretic effect over time.

3. Results

Pyrexia induction using yeast suspension produced a significant increase in rectal temperature in the negative control and paracetamol-treated (PCT) groups when compared to the normal group. As shown in Table 1, rectal temperatures 30 minutes after induction were significantly greater in the negative control (38.6 ± 0.45°C) and PCT (38.6 ± 0.64°C) groups compared with the normal group (36.3 ± 0.80°C), indicating that fever had been successfully induced. This hyperthermic condition was maintained at 60 minutes after induction, as the negative control group attained 38.6 ± 0.39°C, against 37.7 ± 0.59°C for the PCT group, above the 36.7 ± 0.50°C of the normal group. These results are supported graphically by Figure 2, where statistical differences ($p < 0.05$) among groups were noted at these initial time points.

After the administration of paracetamol at time 0 through oral administration, there was a progressive reduction in body temperature in the PCT group. The

Table 1. Rectal temperature responses in rat model of pyrexia before and after paracetamol treatment

Treatment Group	Rectal temperature (°C)							
	Before pyrexia induction	After pyrexia induction		After pyrexia induction and paracetamol administration				
		30 minutes	60 minutes	0 minutes	90 minutes	120 minutes	150 minutes	180 minutes
Normal Group	36,5 ± 0,36	36,3 ± 0,80	36,7 ± 050	36,5 ± 0,29	36,7 ± 0,19	36,6 ± 0,62	36,9 ± 0,56	36,6 ± 0,13
Pyrexia-induced untreated group	36,4 ± 0,18	38,6 ±0,45	38,6 ± 0,39	38,4 ± 0,69	37,8 ± 0,44	37,5 ± 0,78	37,0 ± 0,49	36,5 ± 0,33
Pyrexia-induced Paracetamol-treated group	36,7 ± 0,29	38,6 ± 0,64	37,7 ± 0,59	36,7 ± 0,21	36,5 ± 0,34	36,5 ± 0,64	36,3 ± 0,24	36,3 ± 0,32

PCT group exhibited a notable reduction in the mean temperature (36.5 ± 0.34°C) at 90 minutes post-treatment, while the negative control group still manifested a high temperature of 37.8 ± 0.44°C. The control group was kept at 36.7 ± 0.19°C, meaning that the antipyretic therapy had downregulated the pyretic response to almost normothermia. These differences were statistically significant ($p < 0.05$), indicating the early onset of paracetamol's antipyretic action, as further illustrated in Figures 1 and 2.

At later time points—120, 150, and 180 minutes of administration—group temperature differences started to decline. By 120 minutes, normal (36.6 ± 0.62°C) and PCT (36.5 ± 0.64°C) group temperatures had merged, whereas negative control group temperature decreased regularly to 37.5 ± 0.78°C. By 150 and 180 minutes, the temperatures of all groups had moved towards baseline with no statistically significant differences, indicating either loss of drug activity or inherent thermoregulatory recovery.

These trends over time are very clearly illustrated in Figures 1 and 2, representing the pharmacodynamic effect of paracetamol and the self-limiting nature of the fever when left untreated.

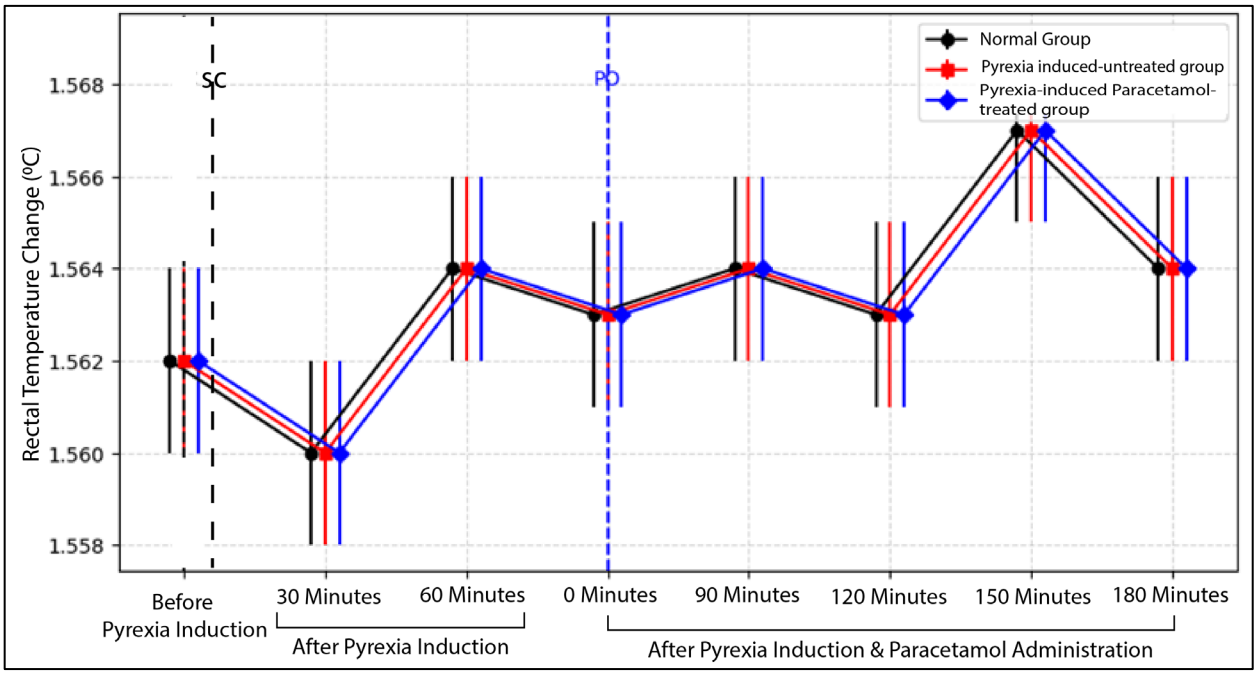

Figure 1. Time-course of rectal temperature in rats following yeast-induced pyrexia and therapeutic intervention with paracetamol

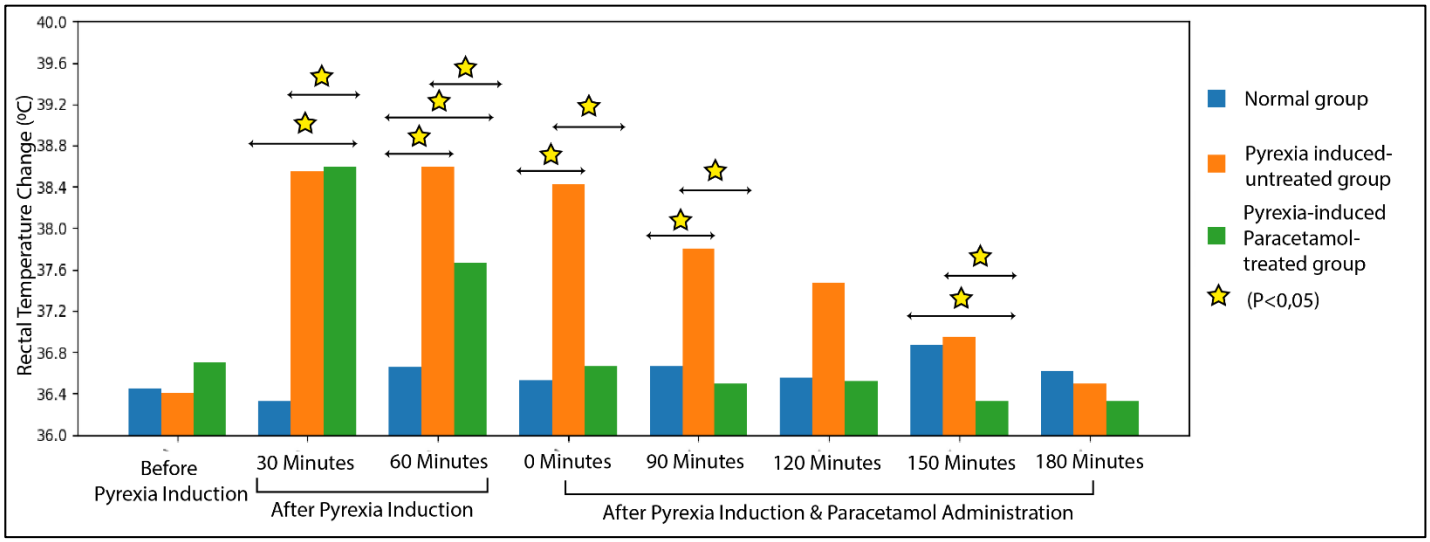

Figure 2. Comparative analysis of rectal temperature in normal, pyretic, and paracetamol-treated rats at defined time points following yeast-induced pyrexia

4. Discussion

This antipyretic test was conducted according to a protocol from existing models, with adaptations to optimize the induction of pyrexia and the evaluation of antipyretic activity in rats.[7,8] This study modified the protocols to develop a specified model of pyrexia that separates antipyretic effects from anti-inflammatory. It is an important distinction, since the traditional models using yeast or carrageenan typically combine inflammatory and febrile responses into one because of common prostaglandin-mediated mechanisms via the production of prostaglandin E2.[12] Therefore, this study used *Saccharomyces cerevisiae* for pyrogen inducer.

Though such models are useful for dual-use assessment, they might not maximally describe the antipyretic agent pharmacodynamics. Rats remain a widely accepted model in antipyretic research due to their cost-effectiveness, ease of handling, and alignment with ethical considerations in animal experimentation. Therefore, the current research highlights the need for a model of specific design to isolate the febrile response with minimal inflammatory confounding factors. Further experiments targeting only the thermoregulatory axis would also be in support of this refinement and increase the predictive validity of preclinical antipyretic screening.

Several preclinical models have been established for the induction of pyrexia, utilizing various immune mechanisms to replicate febrile responses. Of those, Saccharomyces cerevisiae yeast has become an important candidate based on its reproducibility and ability to induce a systemic and controlled febrile condition.[13] In contrast to lipopolysaccharide (LPS), a highly potent endotoxin of Gram-negative bacterial membranes that signals via Toll-like receptor 4 (TLR4) to cause a vigorous pro-inflammatory response by NF-κB and MAPK signaling pathways, yeast β-glucan stimulates macrophage cells' Dectin-1 receptors. Although this also activates NF-κB and MAPK pathways, it activates a less pro-inflammatory cytokine profile with more IL-10 and less pro-inflammatory cytokines TNF-α and IL-6.[14,15] In comparison to cytokines, which mediate their effects via established signal transduction pathways (e.g., JAK-STAT) and tend to induce specific immune responses, yeast induction is a more general inflammatory stimulus for the identification of antipyretics.[16]

Immune response to S. cerevisiae is thus less severe than to LPS, with the benefit of mimicking clinical febrile infection without danger of inducing overwhelming systemic inflammation. This renders it especially suitable for the pharmacological screening of antipyretics, for which reproducibility and moderate, prolonged pyrexia are preferred. Not only is the yeast model a very good model of fever induction, but it also exhibits very human-like immunomodulatory responses, further contributing to its translational potential as a drug screen model.[16]

Several studies have been conducted to evaluate the fever-inducing effects of *Saccharomyces cerevisiae* on rats as an antipyretic research model. The first study examined the body temperature rhythm over 24 hours after the injection of Saccharomyces cerevisiae in Wistar rats. The results showed that yeast-induced fever varied depending on the timing of administration, with a significant shift observed in the peak phase of body temperature.[17] The observation duration in this study involved recording body temperature every hour over a 24-hour period. Another study characterized the fever model in rat pups induced by yeast, with body temperature

monitoring up to 5 hours after induction. The results demonstrated a significant increase in body temperature, which was then used to evaluate the antipyretic effects of the test compound. The body temperature was measured periodically for 5 hours after fever induction.[16] Meanwhile, another study evaluated the antipyretic effects of β-glucan isolated from Saccharomyces cerevisiae in rats induced with fever by LPS. The findings showed that β-glucan had a significant antipyretic effect, with a reduction in body temperature observed up to 6 hours after the administration of β-glucan.[15] The observation duration in this study extended up to 6 hours after the β-glucan administration.

In another previous protocol, *Saccharomyces cerevisiae* was used as the pyrogenic agent at 15% in aqueous suspension and was injected subcutaneously at 10 mL/kg body weight. Antipyretic drug or reference standard drug was usually administered 18 hours after induction, and rectal temperature was recorded at 30–60 minutes intervals to analyze the febrile pattern and pharmacological effect.[18] In the present study, the model was improved by doubling the concentration of yeast to a 40% aqueous suspension without altering the volume per kilogram body weight. This was based on the need for a more intense and consistent febrile response. Our results validate that this modified dose consistently causes pyrexia and justify its further application in antipyretic screening models because of its reproducibility and systemic action in producing a sustained febrile state.

In this study, we selected a paracetamol dose of 150 mg/kg based on previous research that has shown this amount to be both effective and well-tolerated in rat models of fever.[7,8] This dose is commonly used in antipyretic studies because it reliably produces a measurable reduction in body temperature within a short timeframe. During our 180-minute observation period, we did not observe any signs of distress, abnormal behavior, or other adverse effects in the treated animals. These findings suggest that the dose was not only effective but also safe for use in our refined yeast-induced pyrexia model.

Nevertheless, the current study did not measure the antipyretic biomarker to ensure the febrile response after pyrexia induction. This finding was limited to exploring a comprehensive understanding of the molecular mechanisms of action involved in hyperthermia conditions.[7]

Distinguishing true antipyretic activity from general anti-inflammatory effects remains a key challenge in preclinical drug development. Biomarker-guided validation offers a targeted approach, with prostaglandin E2 (PGE2) serving as the most reliable fever-specific marker due to its central role in hypothalamic thermoregulation. Inhibition of PGE2 directly correlates with antipyretic efficacy, independent of systemic inflammation.[9,10] Hypothalamic metabolic markers—such as N6-(1,2-dicarboxyethyl)-AMP, GMP, IMP, and UDP-D-galactose—further reflect fever-specific neurochemical activity, while brain metabolites linked to ellagic acid offer insight into central temperature regulation.[1,10] In contrast, classic inflammatory markers (e.g., cytokines, COX-2, TXB2) lack specificity for fever and are insufficient as sole indicators.[11] Future antipyretic studies might incorporate PGE2 and hypothalamic metabolites to strengthen mechanistic clarity, distinguish antipyretic effects from anti-inflammatory action, and improve the predictive value of preclinical models.

5. Conclusion

The present study established a refined *Saccharomyces cerevisiae*-induced pyrexia model in Sprague Dawley rats, which had the capacity to reliably induce a febrile response and discriminate between the antipyretic and anti-inflammatory actions of pharmacological agents. Injection of a 40% yeast suspension enhanced the consistency of fever without causing excessive systemic inflammation, thereby enhancing the specificity and translational value of the model. Following investigations using biomarkers specific for novel antipyretic screening drugs are suggested to improve mechanistic understanding and model predictability towards preclinical antipyretic drug discovery.

6. Author Contribution

BWKW, THS, and ARK conceived the original idea and provided overall supervision of the project. OAP, R, NRNR, FYP, HNNAB, DSPW, RK, ODU, NMA, and FAG conducted the experiments and collected the data. OAP and BWKW performed data analysis and wrote the manuscript.

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