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Original Research Article

A COMPARATIVE EVALUATION OF SUBCUTANEOUSLY ADMINISTERED PYRETIC AGENTS IN SPRAGUE-DAWLEY RATS: EFFICACY AND PRACTICAL CONSIDERATIONS FOR ANTIPYRETIC SCREENING

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ABSTRACT

Selecting an appropriate pyrexia model is a critical first step in screening novel antipyretic compounds. While several agents are known, a direct comparison of their efficacy and time-course profiles is lacking, leading to potential inefficiencies in research design. This study aimed to compare the pyretic activities of four agents—turpentine, formalin, carrageenan, and brewer's yeast—in a rat model to determine the most suitable agent for antipyretic evaluation studies. A total of 25 Sprague-Dawley rats were randomly assigned to 5 groups, with 5 rats per group. Fever was induced by administering a subcutaneous injection of 2mL/Kg turpentine (100%), formalin (5%), carrageenan (1%), brewer's yeast (15%), and normal saline (control), at the nape of the neck. Body temperature was measured non-invasively at the nape, ear, and abdomen over 540 minutes. All pyrogenic treatments induced a significant increase in temperature above baseline, peaking around 350 minutes. Formalin elicited a significant ($p < 0.05$) pyretic response at all measurement sites (nape, ear, abdomen). Turpentine induced significant pyrexia at the nape and abdomen, while carrageenan and brewer's yeast showed significant effects only at the administration site (nape). Notably, the control group also exhibited a gradual temperature rise, attributed to stress from sleep deprivation. Formalin injection also caused significant localized tissue damage. While all tested agents can induce fever, their efficacy and site of action vary. Formalin and turpentine are the most robust systemic pyrogens. However, formalin's significant tissue toxicity may limit its utility. The observed stress-induced hyperthermia in controls underscores the necessity for carefully controlled experimental conditions. Researchers should select pyretic models based on the desired systemic versus localized response and consider animal welfare implications.

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INTRODUCTION

Fever, or pyrexia, is a complex physiological response characterized by an elevated body temperature above the normal range, mediated by the hypothalamic thermoregulatory center [1]. Fever is a significant characteristic of the acute-phase response to infection, inflammation, or tissue injury [2]. The febrile response begins when exogenous pyrogens, such as microbial products, activate immune cells to release endogenous pyrogens – specifically the pyrogenic cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha

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(TNF-α) [3]. These pyrogenic cytokines promote the synthesis of prostaglandin E2 through the cyclooxygenase (COX) and microsomal prostaglandin E synthase-1 (mPGES-1) pathway. Prostaglandin E2 subsequently acts on EP3 receptors in the median preoptic nucleus of the hypothalamus, resulting in the development of fever [4-6].

In preclinical research, evaluating potential antipyretic drugs relies on robust, reproducible animal models of fever. Several exogenous agents are routinely used to induce pyrexia in laboratory rodents. Notable pyrogens include Brewer's yeast (*Saccharomyces cerevisiae*) - a classic model, which induces fever by provoking a systemic inflammatory response with elevated plasma cytokines [7], and turpentine - a distilled pine resin, known for its ability to induce a strong local inflammation and a systemic febrile response that is less susceptible to tolerance development [8-10].

Aside from the aforementioned, there are pyrogenic agents, such as carrageenan and formalin, commonly used in pain and inflammation research, that have not been thoroughly validated as pyretic models. Carrageenan is a sulfated polysaccharide and the gold standard for inducing paw edema via a complex process involving pro-inflammatory mediators - histamine, bradykinin, and prostaglandins [11]; however, its efficacy as a systemic pyrogen is undefined. Similarly, formalin, widely used in the formalin test for nociception, produces a combination of neurogenic and inflammatory pain [12] and edema [13], yet has limited documentation of its ability to elicit a consistent febrile response.

This gap in the literature creates a challenge for researchers, who may spend considerable time and resources optimizing pyrexia models. Therefore, a direct, comparative assessment of these agents under standardized conditions is needed. This study aims to evaluate and compare the pyretic efficacy, time-course of pyrexia, and practical implications of four agents—turpentine, formalin, carrageenan, and brewer's yeast—in Sprague-Dawley rats, to provide a clear evidence base for model selection in antipyretic drug discovery.

MATERIALS AND METHODS

Equipment

Electronic precision balance (5000g x 0.01g Digital Scale Accurate Electronic Balance, Shanghai Ruishan Electronic Technology Co., Ltd, China), Digital high precision electronic balance (JOANLAB Equipment Co., Ltd, China), digital infrared thermometer (FLUKE-62 Max+, Fluke Corporation, USA), animal plastic cages, syringes and needles (Agary Pharm. Ltd, Lagos, Nigeria).

Chemicals and Reagents

Turpentine (steam-distilled), Formalin (37% stock), Lambda-carrageenan, and Brewer's yeast (Saccharomyces cerevisiae) were procured from Sigma-Aldrich Solutions (Merck KGaA, Darmstadt, Germany). Normal saline (NaCl 0.9%, Fidson Healthcare Plc, 268 Ikorodu-Ososun Road, Obanikoro 100232, Lagos, Nigeria) was used as the vehicle and control.

Animals and Housing

Twenty-five male Sprague-Dawley rats (weighing 162–317g) were sourced from the animal house in the Department of Pharmacology, University of Calabar. The animals were housed in groups within plastic cages and maintained under standard laboratory conditions (temperature: 29–31°C; relative humidity: 60–70%, and a 12 h light/dark cycle). They were provided unrestricted access to standard rodent feed and water and allowed to acclimatize for 2 weeks. The study protocol received ethical approval (420PHA1125) from the Faculty Animal Research Ethics Committee (FAREC-FBMS) at the University of Calabar, and all experimental procedures complied with the OECD guidelines.

Experimental Design

The rats were randomly assigned to five groups, each group containing five rats, and treated as follows: group 1 (Control) received 2mL/Kg normal saline; group 2 were administered 2mL/Kg of 100% turpentine; group 3 were given 2mL/Kg 5% formalin; group 4 received 2mL/Kg 1% w/v carrageenan, while group 5 were administered 2mL/Kg 15% aqueous suspension of brewer's yeast. All substances were administered subcutaneously at the nape of the neck.

Induction of Pyrexia and Temperature Measurement

Pyrexia was induced according to the groupings above. The basal temperature of each rat was recorded prior to injection. Subsequently, body temperature was measured non-invasively using a calibrated digital infrared thermometer (FLUKE-62 Max+) at three sites: the nape of the neck (injection site), the ear pinna, and the dorsal abdominal region. Measurements were taken at 20-minute intervals for

the first 2 hours, then at 30-minute intervals for the remaining 7 hours, for a total of 540 minutes.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Prism 5 for Windows, version 5.01; GraphPad Software, Inc., San Diego, CA). Data were expressed as mean ± standard error of mean (SEM). Two-way Analysis of Variance (ANOVA) followed by Bonferroni's post hoc test was used to determine statistically significant differences among means. $P < 0.05$ was considered statistically significant.

RESULTS

Pyretic Response Across Measurement Sites

The temporal profiles of temperature changes at the nape, ear, and abdomen showed a common trend across all pyrogen-treated groups: a lag phase of approximately 100-150 minutes, followed by a steady increase in temperature, peaking around 300-350 minutes post-injection, then gradually declining.

Nape Temperature:

All four pyrogenic agents induced a significant increase in temperature at the nape injection site. Pyrexia onset occurred approximately 240 minutes after administration, as illustrated in Figure 1.

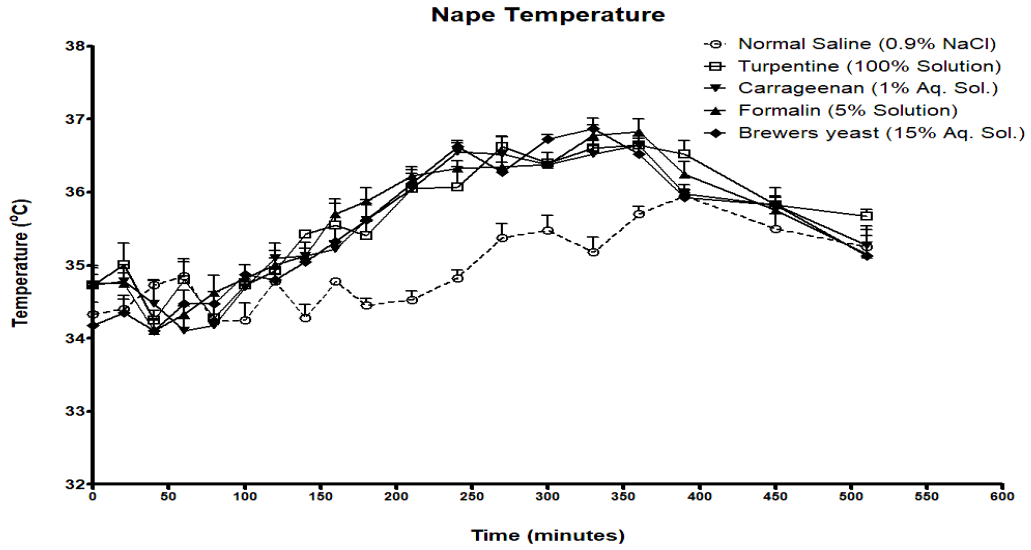


Figure 1. Nape temperature measurements in groups of rats administered with exogenous pyrogens.

Ear Temperature:

Only the formalin-treated group showed a significant increase in ear temperature. Turpentine, carrageenan, and brewer's yeast did not produce a statistically significant elevation at this site (Figure 2).

Abdominal Temperature:

We observed an increase in the body temperature in the formalin ($p < 0.01$) and turpentine ($p < 0.05$) groups. In contrast, carrageenan and brewer's yeast failed to induce a significant temperature rise at the abdominal site, as depicted in Figure 3.

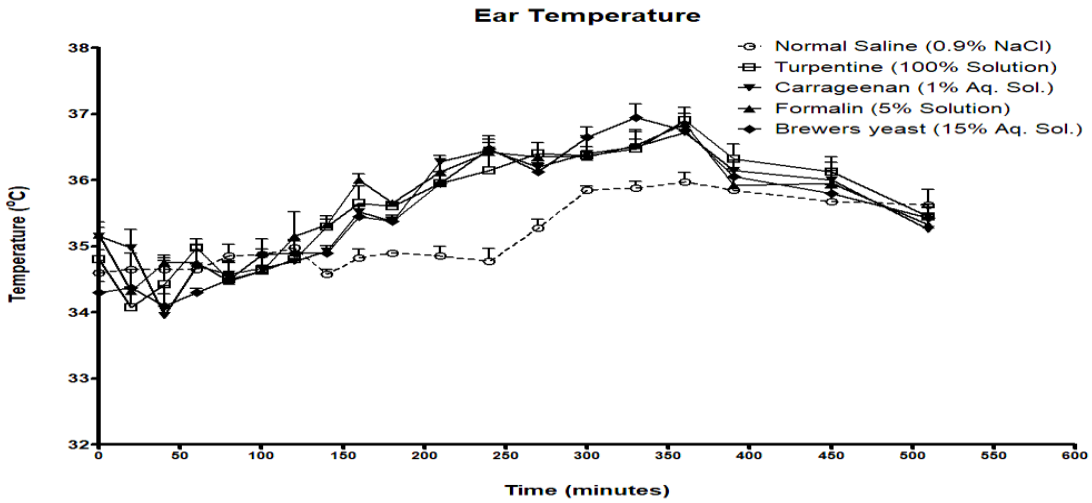


Figure 2. Ear temperature measurements in groups of rats administered with exogenous pyrogens.

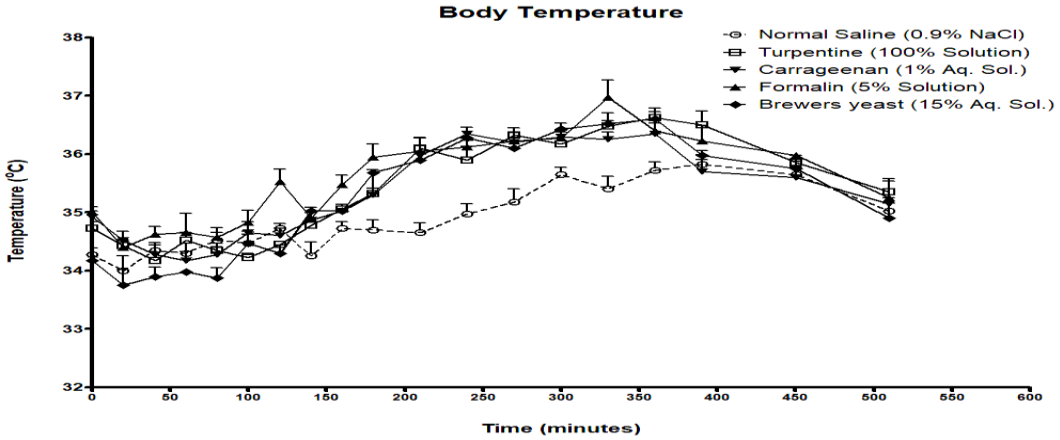
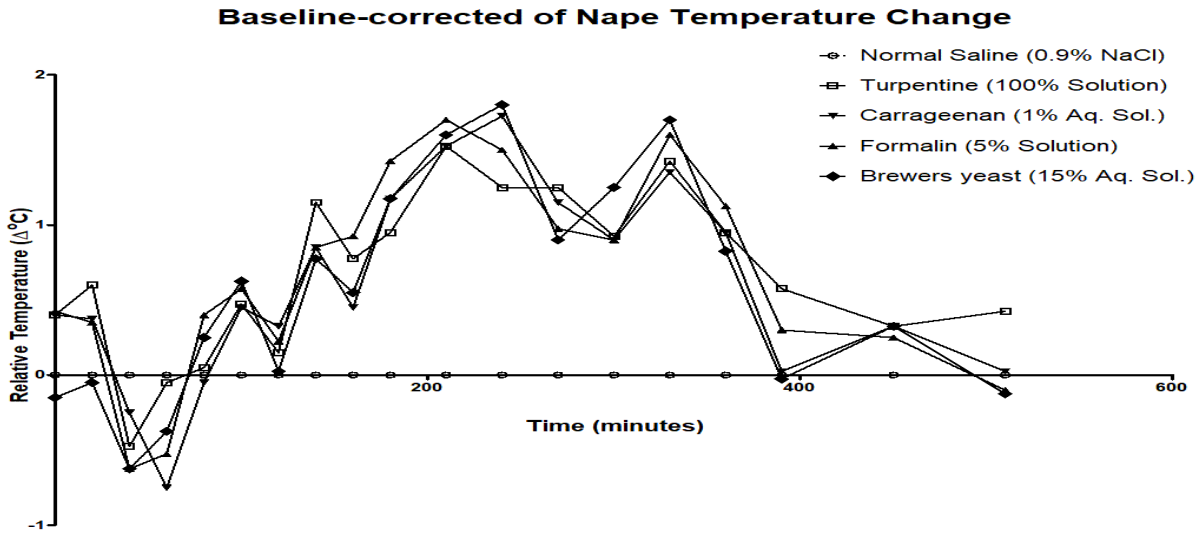
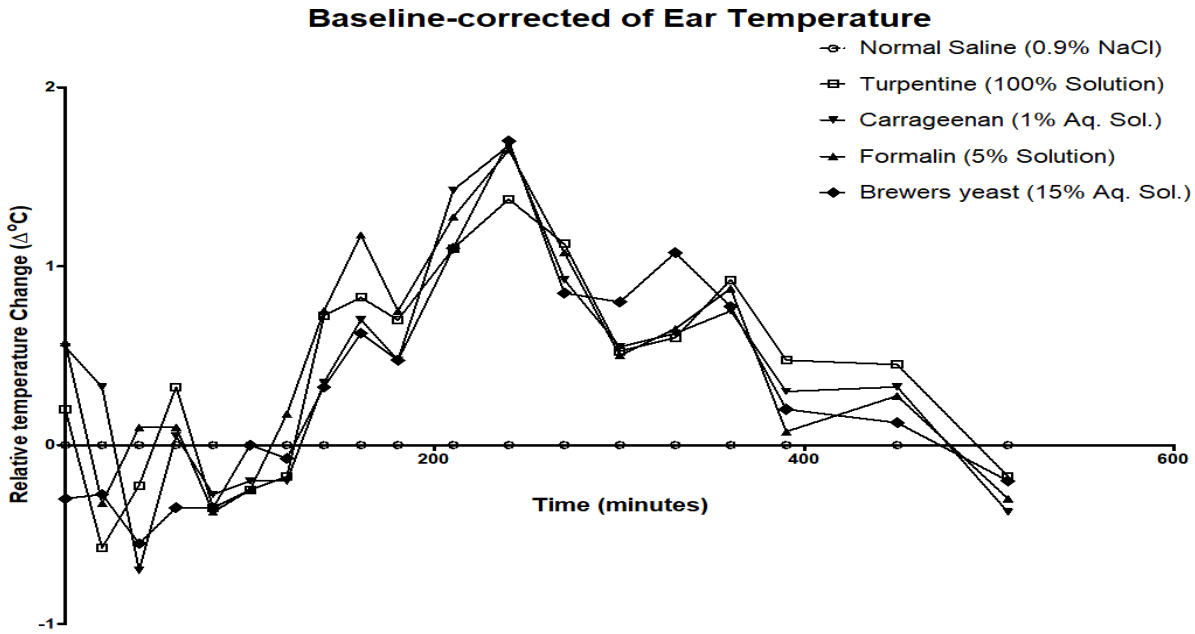


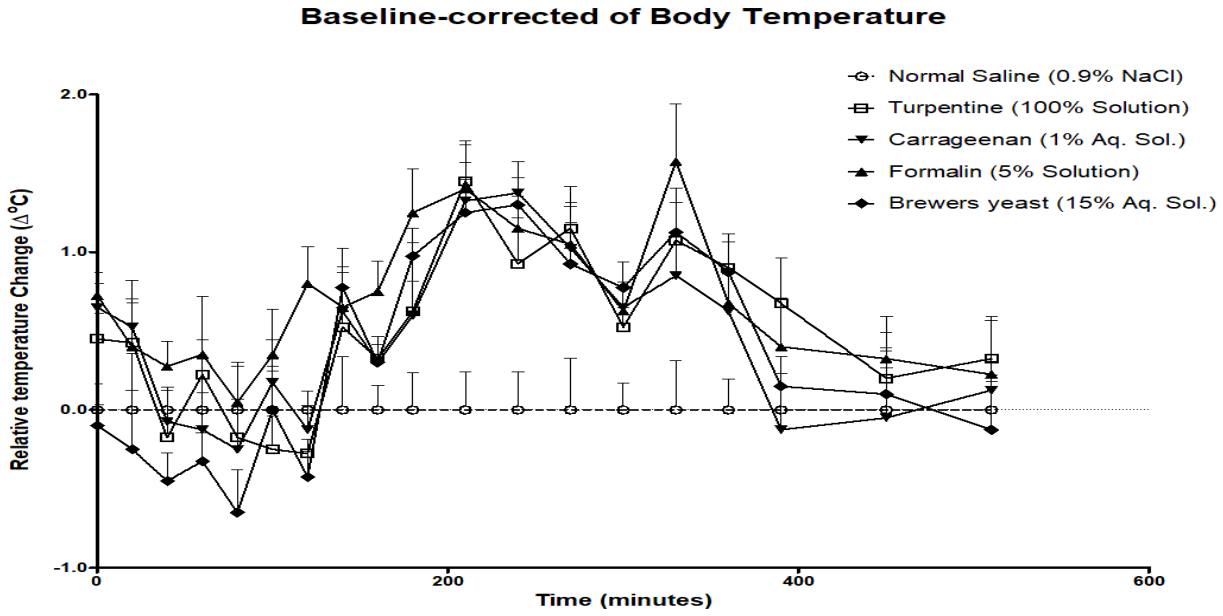
Figure 3. Body temperature measurements in groups of rats administered with exogenous pyrogens



(a)



(b)



(c)

Figure 4. Body temperature measurements in groups of rats administered with exogenous pyrogens. Values are presented as a change in body temperature (°C) relative to the baseline temperature (first measurement). nape (a), ear (b), abdomen (c).

Table 1. Temperature changes at different locations of the rat body after administration of different pyrogens (Turpentine, Formalin, Carrageenan and Brewer's yeast)

	Temperature (°C)		
Treatment	**Ear**	**Nape of neck**	**Abdomen**
Normal Saline (0.9% NaCl)	35 ± 0.12	35 ± 0.12	35 ± 0.13
Turpentine (100% Solution)	36 ± 0.19	36 ± 0.19 **	35 ± 0.20
Formalin (5% Solution)	36 ± 0.18	36 ± 0.20 **	36 ± 0.17 **
Carrageenan (1% Aq. Solution)	36 ± 0.19	35 ± 0.19 *	35 ± 0.18
Brewer's yeast (15% Aq. Solution)	35 ± 0.21	35 ± 0.21	35 ± 0.23

Table 2. Statistical Parameters for Pyrogen Temperature Changes Measured at Different Body Parts of Rats

	Turpentine (100% Solution)			**Formalin (5% Solution)**			**Carrageenan 1% Aq. Solution)**			**Brewer's yeast (15% Aq. Solution)**		
	P value	P value summary	(P < 0.05)	P value	P value summary	(P < 0.05)	P value	P value summary	(P < 0.05)	P value	P value summary	(P < 0.05)
EAR	0.08	Ns	No	0.04	*	Yes	0.09	Ns	No	0.20	ns	No
NAPE	0.005	*	Yes	0.01	*	Yes	0.02	*	Yes	0.03	*	Yes
BODY	0.0057	*	Yes	0.003	*	Yes	0.06	Ns	No	0.23	ns	No

Stress-Induced Hyperthermia in the Control Group

A notable finding was the consistent, albeit lower-magnitude, rise in temperature in the normal saline group across all three measurement sites. This hyperthermic response followed a similar temporal pattern to the pyrogen-treated groups.

Local Tissue Reaction

Gross observation of injection sites 24 hours post-administration revealed severe tissue damage in the formalin-treated group, including necrosis and scab formation (Figure 4A-D). In contrast, the turpentine group showed only mild erythema and swelling in one animal (Figure 4E), while carrageenan, brewer's yeast, and saline groups showed no visible tissue damage.

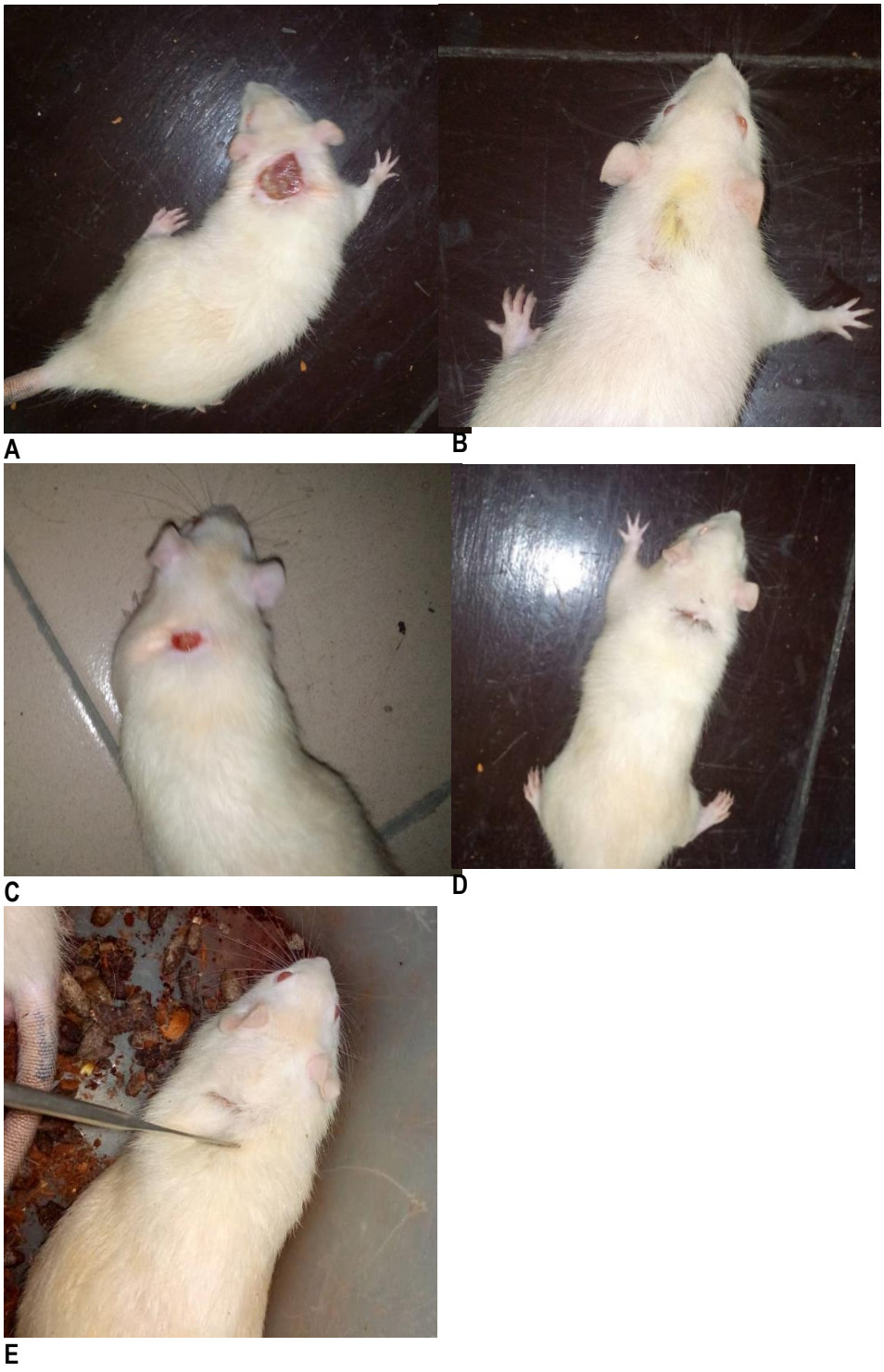


Figure 4. A-D: Wound features in the rat group administered with 5% formalin (2 ml/Kg).
E: The only rat presenting with tissue necrosis in the group that received 100% turpentine (2ml/Kg).

DISCUSSION

This study provides a direct, comparative evaluation of four pyrogens for their ability to induce pyrexia in experimental animals. The results demonstrate clear differences in their efficacy and in the nature of the febrile response elicited, with significant implications for model selection in antipyretic research.

Formalin produced the most pronounced systemic pyrexia, resulting in significant increases in temperature at all three measurement sites. This finding indicates that, in addition to its well-established nociceptive and edematogenic properties, formalin acts as a potent pyrogen. The underlying mechanism likely involves extensive tissue injury at the injection site, triggering a robust local inflammatory response and the subsequent release of endogenous pyrogenic cytokines, including interleukin-1β and tumor necrosis factor (TNF)-α, which act on the hypothalamus to cause fever [4]. This potency, however, is accompanied by a major ethical and practical limitation - namely, the induction of significant and long-lasting tissue necrosis [14], as observed in our study. This tissue damage is a significant welfare concern and a confounding factor in inflammation studies, potentially limiting the use of formalin as a pyretic agent.

Turpentine, a pyrogen commonly used in experimental fever studies [8,15], also induced a significant systemic fever, evident at the nape and abdomen, corroborating its established role as a reliable pyrogen. Turpentine activates monocytes and macrophages to release pro-inflammatory cytokines, which increase prostaglandin E2 levels at the thermoregulatory center of the hypothalamus, leading to increased body temperature [16]. Turpentine is preferred for pyrexia studies in experimental animals because it is resistant to tolerance development [9], and its relatively mild local effects, compared to those of formalin, make it a more suitable candidate for studies requiring a sustained systemic febrile response.

Carrageenan and brewer's yeast produced a significant pyretic effect only at the nape site of administration. This suggests a localized inflammatory reaction rather than a robust systemic febrile response. While brewer's yeast is a classic model for antipyretic screening [17], its effect in this study was localized. The systemic fever typically associated with brewer's yeast may be dose-dependent or require a different route, such as intramuscular injection, to create a sustained reservoir for systemic absorption [7]. Carrageenan's primary use has been to induce localized inflammation and edema [18], and our findings suggest it is a less effective model for studying systemic antipyretics.

A critical observation was the hyperthermic response in the normal saline control group. This phenomenon, often termed stress-induced hyperthermia, is a well-documented regulated rise in body temperature in response to psychological or physiological stressors [19]. Despite using non-invasive temperature measurement to minimize handling stress, the experimental protocol inherently involved sleep deprivation, as the rats (nocturnal animals) were kept awake and monitored during their normal resting phase. This finding underscores the absolute necessity of including a vehicle control group in antipyretic studies to differentiate between drug-induced antipyresis and the natural fluctuation or stress-related changes in body temperature.

According to the criteria for a good pyrogenic model outlined by Tomazetti et al. [17]—including reliability, detectability, rapid onset, sufficient duration, and sensitivity to classic antipyretics—our results position turpentine as a highly effective and practical choice. Formalin, while effective, fails on animal welfare grounds due to its tissue toxicity.

CONCLUSION

This comparative assessment demonstrates that the choice of a pyretic agent profoundly influences the outcome and interpretation of antipyretic studies. Formalin and turpentine are the most effective at inducing systemic fever, but the severe local toxicity of formalin renders turpentine a more suitable and humane option. Carrageenan and brewer's yeast, under the conditions of this study, induced a more localized pyretic response. Furthermore, researchers must account for stress-induced hyperthermia in control animals, a factor that can significantly confound results if not properly controlled. We recommend turpentine as a robust and reliable model for systemic antipyretic evaluation, while cautioning about formalin due to animal welfare concerns. Future studies should investigate the sensitivity of these models to standard antipyretic drugs, such as paracetamol.

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AUTHOR CONTRIBUTIONS

Conceptualization, BASL; methodology, BASL and FKO; software, BASL and FKO; validation, BASL; formal analysis, BASL and FKO; investigation, BASL, FKO and JAB; resources, BASL, FKO, PNW, AGI,

and JAB; data curation, BASL, FKO, JAB, and PNW; writing—original draft preparation, BASL and FKO; writing—review and editing, BASL, FKO, PNW and AGI; visualization, BASL; supervision, BASL and FKO; project administration, BASL.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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