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Website: www.ayujournal.org
DOI: 10.4103/0974-8520.77162
Quick Response Code:

Pharmacological Research

Antipyretic activity of *Guduchi Ghrita* formulations in albino rats

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Abstract

The present pharmacological investigation was undertaken to study the anti-pyretic activity of *Guduchi ghrita* formulations in albino rats against yeast induced pyrexia. Seven groups of six animals were used for the experiment. The yeast induced pyrexia method was standardized first by injecting 12.5% yeast suspension (s.c) followed by recording the rectal temperature at regular intervals. Then the evaluation of anti-pyretic activity of *Guduchi ghrita* formulations was carried out by using this standard procedure. Both the *Guduchi ghrita* samples including vehicle significantly attenuated the raise in temperature after three hours of yeast injection. After 6 and 9 hours of yeast injection also both the *Guduchi ghrita* samples attenuated the raise in temperature in a highly significant manner in comparison to both yeast control and vehicle control groups. The data generated during study shows that both the *Guduchi ghrita* formulations having significant anti-pyretic activity.

Key words: *Guduchi Ghrita*, pyrexia, Brewer's yeast, paracetamol, *Tinospora cordifolia* (Willd.) Miers. medicated ghee.

Introduction

Guduchi [*Tinospora cordifolia* (Willd.) Miers.] has been used in the indigenous system of medicine since the *Vedic* period. It is a very common drug and is quite frequently mentioned in most of the *Samhitas*, *Nighantus*, and *Granthas* for its effectiveness in various diseases. It is considered the best drug in terms of availability, economy, ease of administration, etc. and further, at the dose levels employed clinically it is well tolerated. This plant is used in Ayurveda as single drug in the form of *Swarasa*, *Kalka*, *Kwatha*, *Hima*, *Churna*, and *Ghrita* and also as one of the important ingredients in many other formulations used for treating various ailments. The accepted botanical source of *Guduchi* throughout India is *Tinospora cordifolia* (Willd.) Miers., of the family Menispermaceae. This plant is dioecious, with male and female flowers borne on separate plants. However, during collection of the plant material for medicinal purposes this factor is not given due consideration. It is well established that environmental, agricultural, genetic, storage, and other factors affect the quality of the crude drug. Thus the plant's gender may also impact the therapeutic efficacy of the extract. Many previous studies have shown that in many dioecious species, phyto-constituents are more in the female plant than in the male; thus the source will obviously affect the biological activity.

Ghritas are medicated ghee preparations containing the fat-soluble components of the ingredients. Preparation involves protracted boiling of ghee with prescribed decoctions and a fine paste of the drug to dehydration or near dehydration, whereby the fat-soluble principles are transferred to the *Ghrita* from the drug ingredients or decoctions or expressed juice as the case may be. The *Ghrita* formulation has several advantages over other pharmaceutical preparations in terms of absorption, shelf-life, route of administration, etc.

Guduchi is renowned in Ayurvedic therapeutics for its usefulness in the treatment of *Jwara* (fever).[1] *Guduchi Swarasa* and *Guduchi Kalka* (expressed juice and paste, respectively) prepared from the stem of *T cordifolia* (Willd.) Miers. was used for processing the *Ghrita* (ghee) in this formulation, which is indicated for the treatment of fever.[2,3] Thus prepared formulation of *Guduchi* from male and female plants was subjected to screening for anti-pyretic activity to assess the impact of gender and formulation on expression of pharmacological activity of *Guduchi*.

Materials and Methods

Animals

Wistar strain albino rats of either sex weighing between 140–160 g were used. The animals were obtained from the animal house attached to the pharmacology laboratory of Institute for Post Graduate Teaching and Research in Ayurveda. The

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rats were exposed to natural day and night cycles under ideal ambient laboratory conditions (temperature 22±2°C and humidity 50%–60%). They were fed with Amrut® rat pellet feed (Pranav Agro Industries) and tap water was supplied *ad libitum*.

The experiments were carried out after obtaining permission from the institutional animal ethics committee (approval number – IAEC/3/07-08/01).

Dose

The dose of the test formulations were calculated by extrapolating the human dose (900 mg/kg) to animals based on the body surface area ratio) by referring to the standard table of Paget and Barnes, (1964).[4] The study was carried out using two dose levels, i.e., the therapeutically equivalent dose (TED) of 900 mg/kg and TED × 2 (i.e., 1800 mg/kg).

Study protocol

Animal grouping: Wistar albino rats of body weight ranging from 140 g to 160 g were used as experimental animals. They were divided into seven groups as follows:

- Group I: Yeast control (YC)
- Group II: Vehicle control (VC) (given 900 mg/kg plus yeast injection)
- Group III: *Guduchi Ghrita* prepared from male plant (900 mg/kg plus yeast injection)
- Group IV: *Guduchi Ghrita* prepared from male plant (1800 mg/kg plus yeast injection)
- Group V: *Guduchi Ghrita* prepared from female plant (900 mg/kg plus yeast injection)
- Group VI: *Guduchi Ghrita* prepared from female plant (1800 mg/kg plus yeast injection)
- Group VII: Paracetamol (reference standard) (100 mg/kg plus yeast injection)

Standardization of yeast-induced antipyretic model for the present study

Fever can be induced in experimental animals by intravenous or subcutaneous injection of pyrogens. To evaluate the antipyretic activity of test drugs, the most commonly employed method to induce fever involves injection of lipopolysaccharides (LPS) or brewer's yeast in rabbits or rats.[5]

However, the fact that small animals do not present reliable pyrogen-induced fever makes antipyretics screening difficult and expensive, since the amount of drug necessary for these tests increases proportionally with the size of the animal. The use of different pyrogens in the literature certainly provide an additional source of variation for antipyretic screening results, since it has been reported that lipopolysaccharide-induced fever is dependent on the serotype of its source.[6] In addition, there are several reports of hypothermia, instead of fever, after LPS and yeast administration to rats and mice.[7]

Presently available methods of yeast-induced pyrexia in rats include development of fever usually 9–18 h after yeast injection.[8] It is essential to develop a model in which fever ensues within a few hours (4–5 h) of yeast injection to test the classical antipyretic drug. It is also necessary to have long-lasting fever episodes (up to 24 h) as the onset of therapeutic action and duration of therapeutic action of classical drugs is believed to be more.

Against this background, the yeast-induced pyrexia model was standardized prior to the experiment proper. Since the Charles Foster rats available in the animal house of our institute did not develop pyrexia on injection of Brewer's yeast, this study was carried out on albino rats of the Wistar strain. Young rats (three males and three females) were selected. It is reported that in yeast-induced pyrexia, adult rats develop fever within 9–18 h[7] and that age can influence the response to the pyrogen, which may account for different immune responses.[9,10]

The animals were fasted for 18 h before the commencement of the experiment, but drinking water was provided *ad libitum*. Rectal temperature (T_R) was measured by inserting a lubricated thermostat probe (external diameter 6 mm) 3 cm into the rectum of the animal. The probe was linked to a digital device, which displayed the temperature at the tip of the probe with 0.1°C precision. The values displayed were manually recorded.

Immediately after measuring the initial basal rectal temperature, the animals were injected with brewer's yeast in normal saline (12.5%; 1 ml/100 g body weight, subcutaneously). The rectal temperature changes were recorded every hour up to 12 h, and expressed as the difference from the basal value. Since it has been previously reported that stress related to handling and temperature measurment can alter rectal temperature,[11] these animals were handled carefully so as to minimize the posible stress. The increase in T_R occurred almost at the end of 2 h after yeast injection. This increase was around 2.25 ± 0.08°C (mean ± SEM where n=6) at 3 h, 2.50 ± 0.07°C at 6 h, and 2.73 ± 0.08° C at 9 h. After this time interval, the T_R did not increase steadily up to 12 h; instead, a decrease in T_R was observed in some rats. This standardized method was used for the present study.

The test drugs and reference standard were administered to the respective groups. One hour after drug administration, the yeast injection was given s.c. and the rectal temperature was recorded at the end of the 3rd, 6th, and 9th hours. The rectal temperature of the control groups (yeast control) was compared with rectal temperature of the rats administered the test drugs.

Statistical analysis

The results are presented as mean ± SEM. The difference between the groups was statistically analyzed using the unpaired Student's t test and analysis of variance (ANOVA) followed by Dunnett's t test for all the treated groups (except reference standard group, where only the unpaired Student's t test was applied). $P<.05$ was considered statistically significant. The level of significance was noted and interpreted accordingly.

Results

Table 1 shows data related to the effect of *Guduchi Ghrita* on yeast-induced pyrexia at different time intervals. Yeast injection in experimental animals caused significant rise in body temperature at the various time intervals as recorded rectally with the help of a tele-thermometer. Paracetamol, a well-established antipyretic drug attenuated the rise in temperature to a significant extent at all time intervals. Both the *Guduchi Ghrita* samples including vehicle significantly attenuated the rise in temperature 3 h after yeast injection. After 6 and 9 h of yeast injection also both the *Guduchi Ghrita* samples attenuated the raise in temperature in a highly significant

Table 1: Effect of *Guduchi Ghrita* prepared from male and female plants of *T cordifolia* on yeast-induced pyrexia at various time intervals in albino rats

Actual change in rectal temperature (°C)			
Groups	**3 hours**	**6 hours**	**9 hours**
Yeast control	02.25 ± 0.08	02.50 ± 0.07	02.73 ± 0.08
VC	01.95 ± 0.08^{C}	02.38 ± 0.14	02.61 ± 0.08
MGG TED	01.31 ± 0.08$^{C\phi}$	02.06 ± 0.17$^{C\phi\phi\phi}$	02.15 ± 0.08$^{B\phi\phi\phi}$
MGG TED × 2	01.37 ± 0.08$^{C\phi}$	01.72 ± 0.13$^{C\phi\phi\phi}$	01.75 ± 0.12$^{C\phi\phi\phi}$
FGG TED	01.90 ± 0.12^{A}	02.08 ± 0.15$^{C\phi\phi\phi}$	02.00 ± 0.17$^{C\phi\phi\phi}$
FGG TED × 2	01.13 ± 0.12$^{C\phi\phi}$	01.47 ± 0.10$^{C\phi\phi\phi}$	01.45 ± 0.12$^{C\phi\phi\phi}$
Paracetamol	00.98 ± 00.18***	01.47 ± 00.28**	01.77 ± 00.18***

Values were expressed as mean ± SEM. $^{A}P<.05$, $^{B}P<.01$, $^{C}P<.001$ (Dunnet's multiple *t* test) compared with yeast control, **$P<.01$, ***$P<.001$ (Student's *t* test) compared with yeast control, $^{\phi}P<.05$ $^{\phi\phi}P<.01$, $^{\phi\phi\phi}P<.001$ (Dunnet's multiple *t* test) compared with vehicle control, MGG – Male *Guduchi ghrita*, FGG – Female *Guduchi ghrita*, TED – Therapeutically equivalent dose

manner in comparison to both yeast control and vehicle control groups. The vehicle itself had a moderate antipyretic effect in the initial stages; at the later stages, however, the observed antipyretic activity was only marginal.

Discussion

Fever is a surrogate marker for disease activity in many infectious and inflammatory disorders. According to the classical view, the genesis of fever is induced by inflammatory mediators (i.e., cytokines, namely interleukin-1, interleukin-6, tumor necrosis factor, and others) that are predominantly released by activated peripheral mononuclear phagocytes and other immune cells.[12,13] Due to the fact that direct access of the large hydrophilic cytokine proteins to the temperature-controlling brain structures within the pre-optic/anterior hypothalamic areas is prevented by the blood–brain barrier, the mechanisms described below have been suggested for producing pyrexia.

Cytokines which are transported by the bloodstream could act at sites lacking a tight blood–brain barrier, the so-called circumventricular organs.[14] Alternatively, circulating cytokines could interact with their specific receptors on brain endothelial cells[15] or perivascular cells[16] and thereby stimulate these cells to release pyrogenic mediators into the abluminal brain tissue. It has been proposed that fever-promoting cytokines are transported from the blood into the brain via specific carriers.[17] An assumed manifestation of a febrile response produced by these mechanisms is termed as the humoral hypothesis of fever induction. Within the brain, prostaglandin E_2 (PGE_2), produced by cyclooxygenase (COX)-2, is regarded as the principle downstream mediator of fever[18] acting on thermosensitive or thermointegrative hypothalamic neurons.

Fever is tightly regulated by the immune response. Inflammatory stimuli triggering the generation of pro-pyretic messages provoke the release of endogenous antipyretic substances.[18] PGE_2 is synthesized from arachidonic acid, which is released from cell membrane lipid by phospholipase. Arachidonic acid is metabolized by two isoforms of the COX enzyme, COX-1 and COX-2. COX-1 usually is expressed constitutively and generates prostanoids important for housekeeping functions supporting homeostasis.[19] COX-2, on the other hand, is inducible by inflammatory signals such as the pyrogenic cytokines, IL-1b, TNF, and IL-6, as well as bacterial lipopolysaccharide. Many cells, including synoviocytes, macrophages, endothelial cells, and chondrocytes, have the capacity to rapidly up-regulate the expression of the COX-2 during inflammation.[19] The most likely cell type in the central nervous system responsible for producing PGE_2 is the microvascular endothelial cell, which expresses COX-2 exuberantly after stress. An effective febrifuge might interrupt pyrexogenesis at any step that connects peripheral inflammation with the central production of PGE_2. Stated differently, an antipyretic might blunt peripheral inflammation or depress central pyrogenic signals, or it may affect both. Inhibiting central production of PGE_2 is a well-known mechanism of antipyretic agents, but activated leukocytes and endothelial cells in peripheral areas of inflammation also represent potential drug targets.

Paracetamol is an analgesic but is also an effective febrifuge. It is a poor inhibitor of cyclooxygenase in the presence of peroxides that are found in inflammatory lesions. In contrast, its antipyretic effect may be explained by its ability to inhibit cyclooxygenase in the brain, where peroxide tone is low. Further, it does not inhibit neutrophil activation. In supra-pharmacologic doses it inhibits NF-kB stimulation of inducible nitric oxide synthase.[20]

In the present study, in the yeast control group the rise in temperature was consistent and significant in comparison to the initial values. In the vehicle control group also the rise in temperature was significant; however, the magnitude was slightly less in comparison to that in the yeast control group in the initial stages. Both the *Guduchi Ghrita* samples produced very good antipyretic effect in a dose-dependant manner and the observed effect was almost similar to that in the paracetamol-treated group.

Previous studies by Ikram *et al.*[21] and Leghari *et al.*[22] have reported the antipyretic effect of *Tinospora cordifolia* in Himalayan rabbits. Vedavathy and Rao,[23] showed water-soluble fractions of a 95% ethanolic extract of *T cordifolia* plant to possess significant antipyretic activity (when given orally) against yeast-induced pyrexia. Many authors have ascribed the antipyretic properties of *T cordifolia* to the presence of berberine or other bitter substances.[24]

Conclusion

From the analysis of the data generated in this study it becomes

clear that the dioecious nature of the plant does not influence the expression of the antipyretic activity since no statistically significant difference could be found between male and female plant–derived *Ghritas*. The second point to be noted is that though the vehicle itself exhibits a short-lived mild to moderate antipyretic activity, the test *Ghritas* have significantly higher activity in comparison to the vehicle control. This clearly shows that the active principles present in the plant *per se* possess significant antipyretic activity, which might be supplemented by the vehicle. Further, in an earlier study (Savrikar, 2006)[25] it was observed that the antipyretic activity of the expressed juice *per se* is short lived but when administered in the form of *Ghrita* it is long-lasting. In the present study also a long-lasting antipyretic activity was observed. Thus, the study provides unequivocal evidence of the presence of antipyretic activity in the test *Ghritas*.

In this study no attempt was made to ascertain the mechanism of the observed antipyretic activity. However, it can be suggested that it may be acting through either the peripheral or central mechanism enumerated above. It is also possible that both the mechanisms may be involved. Considering the lipoid nature of the vehicle used it seems that lipid-soluble constituents may be responsible for the observed effect.

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हिन्दी सारांश

अल्बीनो चूहों में गुडुची घृत कल्प का ज्वरघ्न कर्म

अशोक बी. के. बी. रविशंकर पी.के.प्रजापति सविता डी. भट्ट

प्रस्तुत फार्माकोलोजिकल परीक्षण, गुडुची घृत का अल्बीनो चूहों में यीस्ट प्रत्युत्पन्न ज्वर में ज्वरघ्न कर्म हेतु किया गया। परीक्षण में ६ चूहों के सात समूह लिये गये, प्रथम १२.५ % यीस्ट इंजेक्शन देकर, यीस्ट प्रत्युत्पन्न ज्वर की विधि का प्रामाणिकीकरण किया गया एवं नियमित समयांतराल में गुदा प्रदेश का तापक्रम लिया गया। इसी प्रामाणिक विधि से गुडुची घृत का ज्वरघ्न कर्म देखा गया। सहपान सहित दोनों गुडुची घृत कल्पों द्वारा यीस्ट इंजेक्शन के ३ घंण्टे बाद ज्वर का प्रभावी रुप में शमन किया गया। यीस्ट इंजेक्शन देने के ६ एवं ९ घण्टे के बाद गुडुची घृत के दोनों प्रकारों द्वारा यीस्ट कंट्रोल व सहपान वर्ग की तुलना में अतिप्रभावी ज्वरघ्न कर्म पाया गया। अध्ययन से प्राप्त परिणामों द्वारा दोनों प्रकार के गुडुची घृत कल्पों में अतिप्रभावी ज्वरघ्न कर्म देखा गया।