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Original Research Article (Experimental)

In vivo evaluation of antipyretic effects of some homeopathic ultra-high dilutions on Baker's yeast-induced fever on Similia principle

Saeed Ahmad [a, b], Tayyeba Rehman [b, *], Waheed Mumtaz Abbasi [b]

[a] *Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan*
[b] *University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan*

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ABSTRACT

Background: Homeopathy is a controversial alternative system of medicine. The action of homeopathic medicines is considered slow and it is assumed that homeopathic medicines are ineffective in acute conditions such as fever.
Objective: In the present study, effects of 3 homeopathic medicines on baker's yeast induced fever were investigated.
Materials and methods: 42 local strain rabbits were equally divided into 7 groups. Normal saline was orally administered to group 1 (normal control) rabbits without fever induction. Group 2 underwent baker's yeast-induced fever (negative control). Groups 3, 4, 5, 6 and 7 underwent baker's yeast-induced fever and were thereafter treated orally with paracetamol, *Nux vomica* 200C and 1M, *Calcarea phos* 200C and *Belladonna* 200C respectively. Rectal temperature was checked hourly. The abdominal writhing and frequency of loose stools were also monitored. ANOVA was applied for checking statistical significance. $p \leq 0.05$ was considered significant.
Results: The rectal temperature increased significantly ($p < 0.05$) in the negative control group when compared to the normal control. Abdominal writhing and loose stools monitoring showed increased writhing and loose stools frequency of group 2, 3, 6 and 7 rabbits. However, treatment of paracetamol significantly reduced rectal temperature. Group 4 & 5 showed significant reduction of rectal temperature together with abatement of abdominal writhing and loose stools.
Conclusion: N. vomica ultra-high dilutions have normalized rectal temperature and prevented the abdominal writhing and loose stools in baker's yeast-induced fever model of rabbits. It could be due to antidotal activity of *N. vomica* ultra-high dilutions. Therefore, *N. vomica* ultra-high dilutions can be useful antipyretic agents and can treat conditions associated with gastrointestinal symptoms. However, fixed conclusion can't be asserted due to caveat of small sample size.

1. Introduction

Fever is a complex response produced by infectious as well as non-infectious inflammatory conditions and is manifested primarily as elevated body temperature of about 1–4 °C [1]. Hyperthermia and fever may precipitate brain cell damage [2]. Antipyretic drugs such as aspirin, NSAIDs have been developed for use [3] but mostly produce side effects [4–6]. Hence, there is a need for herbal medicines/homeopathic remedies with antipyretic effect and minimum/no side effect to be investigated.

Homeopathy is based on "*Similia similibus curantur*" which implies that treatment is done with something that is able to produce an effect similar to the suffering [7]. Homeopathy is a widely used but controversial alternative system of medicine [8]. The action of homeopathic medicines is considered slower. It is assumed that homeopathic medicines are ineffective in acute conditions such as fever. But anecdotal evidence indicated that various homeopathic medicines are being used for the treatment of fever. The old literature of homeopathy presents *Nux vomica* as an antidotal remedy for large doses of drugs, alcohol and foods [9]. Different researches

* Corresponding author.
E-mail: tayeba.rehman@yahoo.com
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substantiate the antidotal property of *N. vomica* ultra-high dilutions in alcohol intoxication [10–14]. In practice, *N. vomica* is used for various digestive disturbances and Crohn's disease [9]. Moreover, *N. vomica* is among the most frequently prescribed medicines in clinical practice of homeopathy [15].

Belladonna (*Bell*) is a homeopathic medicine for inflammation associated with heat [16]. *Bell* has reported actions viz antibacterial, anti-inflammatory, antiseptic, neurotropic and anti-protozoal [17–21]. In homeopathy, *Calcarea phosphoricum* is mostly used for bone problems [16].

Baker's yeast is commonly used in baking and brewing products [23]. In experimental animals, it induces fever with elevated plasma levels of IL-1b, interferon-c and TNF-α [24–26]. Various studies indicated baker's yeast fever induction to rabbits [27–29] and rats [24,26,30,31]. In current study, rabbits were selected as they develop fever more easily than rats [32]. Moreover, they have docile nature [33]. Baker's yeast is also a common dietary antigen and various antibodies against it are present in patients with Crohn's disease [23]. Crohn's disease may present with ileitis, ileocolitis diarrhea and fever accompanied with right lower quadrant pain [34].

In literature review, the antipyretic activity of ultra-high dilutions of *N. vomica*, *Bell* and *Calc. phos* against baker's yeast induced fever is not reported yet. The present experiments were therefore undertaken to find effects of *N. vomica* (remedy for gastric fever), *Bell* 200C (routine remedy for fever) and *Calc. phos* 200C (remedy has no relation to fever or digestive disturbances) against baker's yeast-induced fever. The secondary objective of study to see effects of all the medicines on gastric symptoms (diarrhea, abdominal writhing etc.).

2. Materials and methods

2.1. Drugs and chemicals

Paracetamol GlaxoSmithKline, Pakistan, Limited; *N. vomica* 1M & 200C, *Belladonna* 200C, *Calcarea phospohoricum* 200C (Dr. Willmar Schwabe GmbH & Co. KG, Germany); Baker's yeast (Rossmoor food products, Karachi, Pakistan); Ethanol (Merck, Germany); Succussed Alcohol 90% (Masood Homeopathic Pharmaceuticals, Pakistan); Normal Saline (Shazeb Pharmaceutical Industries Ltd); Flagyl-S 200 mg/5 ml Oral Suspension (SANOFI, Ireland).

2.2. Apparatus

Digital thermometer (Medisign MANA & CO Pakistan), Syringes (B.D Singapore).

2.3. Animals and housing conditions

Animals (male and female) used in this study were local strain rabbits (1.5–2 kg). Animals were housed in animal house of Khawaja Fareed Campus, Faculty of Pharmacy and Alternative Medicine, The Islamia University Bahawalpur. Before the start of the experiment, animals were acclimatized to animal house for seven days. Environmental conditions were maintained throughout the study period (12 h light/dark cycles, 23–25 °C and 50–55% humidity). They were provided with standard food and tap water *ad libitum*. They were fasted 24 h before the experiment but were given free access to water. The experiment complies with the declarations of Institute of Laboratory Animal Resources, Commission on Life Sciences [35]. The experimental protocol of current study was approved by Pharmacy Research Ethics Committee via notification number 88-2015/PREC. The manuscript complied with the ARRIVE guidelines [36].

2.4. Experimental design and procedure

In the current study, grouping of animals, drug administration, observations and analysis of results were conducted blind. Animals were divided into seven groups and each group contained 6 rabbits. Sample size was calculated by "resource equation" method [37]. All the rabbits were weighed and dosages of baker's yeast and paracetamol were adjusted according to each rabbit. Rectal temperature was monitored with a digital thermometer. Fever was induced according to the method of Tomazetti [26]. All the groups (except normal control) were treated with 135 mg/kg baker's yeast (*Saccharomyces cerevisiae*) suspension intraperitoneal for fever induction. Normal control group received normal saline intraperitoneal injection. Rectal temperature was checked after 4 h of intraperitoneal injection. 0.5–1.5 °C increase of temperature was considered as induced fever in rabbits [28]. After fever induction, the rabbits were given medicines according to their corresponding groups. Group 2 was negative control and it received 90% succussed alcohol mixed in 5 cc distilled water. Group 3 was standard control and it received paracetamol 150 mg/kg orally [28]. Groups 4 and 5 were given *N. vomica* 1M and 200 respectively. Groups 6 and 7 received *Calc. phos* 200C and *Bell* 200C respectively. All the homeopathic medicines and 90% succussed alcohol (vehicle used for homeopathic medicine as mentioned on purchased homeopathic potencies) were given orally in distilled water. For this purpose, 5 drops of each medicine were mixed in distilled water and 5 cc of this medicine containing distilled water was administered orally to rabbits of respective groups. *N. vomica* was administered orally in current study because a previous study showed its effects through oral receptors [10]. The other medicines were also given orally as the effect of route of administration of these medicines has not been evaluated previously. Moreover, minimum dose of ultra-high dilutions was administered to avoid medicinal aggravation. Rectal temperature was recorded hourly for 6 h after medicine administration. The animals were treated and assessed according to their group number mentioned (Fig. 1). Primary outcome was the assessment of temperature reduction that was checked with the help of digital thermometer. The secondary outcome measure was digestive symptoms that was assessed by appearance of loose stools.

2.5. Statistical analysis

Results of the current study were analyzed by IBM SPSS 20.0 software (statistics.v20_32bit_oxava.com). All the analyses were blinded. Temperature readings were expressed as Mean ± Standard Error of Mean (S.E.M) of six readings. The test applied for analysis of data was Analysis of variance (ANOVA) followed by post hoc test. ANOVA for each time point was used for comparison between groups. Fischer LSD (Least significant difference) post hoc test was applied only if ANOVA was significant. For comparison of baseline data to follow up, Mann-Whitney U test was applied. $p \leq 0.05$ was taken as significant (Table 1).

3. Results

Baker's yeast administration caused fever in all the rabbits, as indicated by a drastic increase in temperature (Table 2). All the animals were returned to animal house for reuse in experiments.

3.1. Effect of normal saline intraperitoneal injection

As shown in Table 2, normal saline treatment caused no temperature variation in rabbits till the end of experiment.

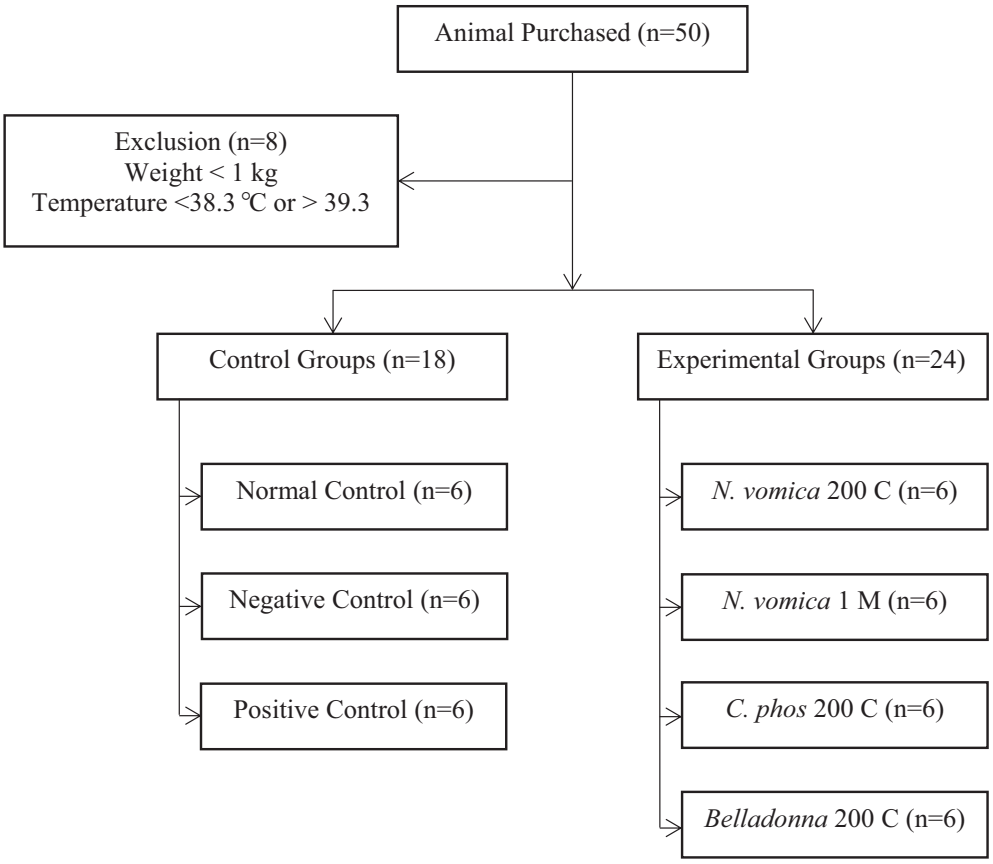

Fig. 1. Animals grouping. Animals were acclimatized to animal house conditions for 7 days. The person blind to the experiment randomly made 7 groups.

3.2. Effect of vehicle on fever induced by Baker's yeast

The negative control group showed a continuous increase in temperature till the 8th hour from the time of yeast administration. At 0 h, normal mean temperature of the group was 38.6 ± 0.1 °C. Up till 8th hour, a temperature rise of 1.2 °C was observed. The temperature started to decline later and about 0.7 °C decrease was observed in last 2 h of experiment. At the end of experiment, 0.6 °C temperature was still raised as compared to baseline temperature (Table 2). Two rabbits suffered from diarrhea and recovered after treatment of Flagyl syrup.

3.3. Effect of paracetamol on fever induced by Baker's yeast

An increase of 0.9 °C was observed after 4th hour of yeast administration while administration of paracetamol decreased the temperature by 0.9 °C in the first hour of medicine administration ($p < 0.002$). In the succeeding two hours, the temperature further decreased by 0.2 °C ($p < 0.001$). In the last two hours of study, a slight increase in temperature (0.2 °C) was observed (Table 2). The temperature at the end of experiment was similar to baseline temperature. Two rabbits in this group developed loose watery stools in 6th hour of study. Flagyl syrup was administered orally and loose stools turned to normal stools.

3.4. Effect of N. vomica 1M on fever induced by Baker's yeast

The temperature increased by 0.8 °C after 4th hour of yeast administration. After medicine administration, a fast and long term decrease of 1 °C was observed that remained almost constant till 6th hour of the experiment ($p < 0.001$). The temperature at the end of experiment was 0.2 °C less than the base line temperature (Table 2). Stools consistency was normal till the end of experiment.

3.5. Effect of N. vomica 200C on fever induced by Baker's yeast

The temperature increased by 0.8 °C after 4 h of yeast administration. After medicine administration, an immediate marked decrease of 0.8 °C was observed that was incessant till 6th hour of

Table 1
Baseline characteristics of different groups.

Baseline characteristics	Baseline data of different groups.						
	Normal control	Negative control	Paracetamol	*Nux v* 200C	*Nux vomica* 1M	*Calc. phos* 200C	*Bell* 200C
Body weight (kg) (Mean ± S.E.M)	1.3 ± 0.08	1.3 ± 0.08	1.3 ± 0.07	1.3 ± 0.09	1.3 ± 0.08	1.3 ± 0.1	1.3 ± 0.07
Temperature °C (Mean ± S.E.M)	38.9 ± 0.08	38.8 ± 0.12	38.7 ± 0.15	38.8 ± 0.08	38.9 ± 0.08	38.8 ± 0.12	38.7 ± 0.15

*There were no significant differences between any of the groups on any of the two variables.

Table 2
Temperature readings in different hours of study in different groups.

Groups	Treatment dosage	Rectal temperature °C (before treatment)		Rectal temperature °C after medicine administration (after treatment)					
		0 hour	4th hour	5th hour	6th hour	7th hour	8th hour	9th hour	10th hour
Normal control		38.9 ± 0.08	38.9 ± 0.12	38.9 ± 0.08#	38.9 ± 0.07#	38.9 ± 0.15#	38.9 ± 0.06#	38.9 ± 0.08#	38.9 ± 0.08**
Negative control	5 drops in 5 cc distilled H_2O	38.8 ± 0.12	39.6 ± 0.15	39.6 ± 0.15	39.9 ± 0.14	40.0 ± 0.14	40.1 ± 0.08	39.7 ± 0.19	39.4 ± 0.12
Paracetamol	150 mg/kg	38.7 ± 0.15	39.6 ± 0.15	38.7 ± 0.14#	38.5 ± 0.13#	38.5 ± 0.13#	38.7 ± 0.11#	38.7 ± 0.14#	38.7 ± 0.16#
Nux v 200C	5 drops in 5 cc distilled H_2O	38.8 ± 0.08	39.6 ± 0.07	38.8 ± 0.05#	38.8 ± 0.06#	38.8 ± 0.07#	38.8 ± 0.09#	38.8 ± 0.13#	38.8 ± 0.07**
Nux v 1M	5 drops in 5 cc distilled H_2O	38.9 ± 0.08	39.7 ± 0.12	38.8 ± 0.04#	38.6 ± 0.10#	38.7 ± 0.08#	38.7 ± 0.09#	38.7 ± 0.10#	38.7 ± 0.08#
Belladonna 200C	5 drops in 5 cc distilled H_2O	38.7 ± 0.15	39.6 ± 0.09	39.6 ± 0.08^	39.6 ± 0.06^	39.6 ± 0.05^	39.5 ± 0.03^	39.4 ± 0.19^	39.2 ± 0.06^
Calc. phos 200C	5 drops in 5 cc distilled H_2O	38.8 ± 0.12	39.6 ± 0.06s	39.8 ± 0.05^	39.8 ± 0.08^	39.9 ± 0.14^	40.1 ± 0.16^	39.7 ± 0.06^	39.5 ± 0.09^

Note: ^ $p \geq 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, #$p \leq 0.001$ compared to negative control. Values are expressed as mean ± S.E.M (N = 6), 0 h reading is normal temperature of rabbits before yeast induction, before treatment 4 h reading is after fever induction reading. 5th to 10th hour readings are after medicine administration readings.

the experiment ($p < 0.001$). The temperature at the end of experiment matched the baseline temperature (Table 2). Stools consistency was normal for the whole period of experiment in this group.

3.6. Effect of Belladonna 200C on fever induced by Baker's yeast

Belladonna 200C showed a continuous increase in temperature till the 7th hour of yeast administration. At 0 h, normal mean temperature of the group was 38.7 ± 0.1 °C. A temperature rise of 0.9 °C was observed after yeast administration that remained same till 7th hour of experiment. The temperature started to decline later and about 0.4 °C decrease was observed in last 3 h of experiment. At the end of experiment, 0.5 °C temperature was still raised as compared to baseline temperature (Table 2). Three rabbits in this group suffered from loose stools and recovered after treatment of Flagyl syrup.

3.7. Effect of Calc. phos 200C on fever induced by Baker's yeast

Calc. phos 200C showed a continuous increase in temperature till the 8th hour from the time of yeast administration. At 0 h, normal mean temperature of the group was 38.8 ± 0.1 °C. Up till 8th hour, a temperature rise of 1.3 °C was observed. The temperature started to decline later. About 0.6 °C decrease was observed in last 2 h of experiment. At the end of the experiment, a rise of 0.7 °C was observed as compared to baseline temperature (Table 2). Two rabbits in this group suffered from loose stools andrecovered after treatment of Flagyl syrup.

4. Discussion

Homoeopathy is a widely used complementary and alternative system of medicine [8]. Homoeopathy faces many criticisms, the most common condemnation is about biological activities of homoeopathic ultra-high dilutions beyond Avogadro' s number [38]. The present study reported the antipyretic effects of *N. vomica* ultra-high dilutions (beyond Avogadro's number of molecules) in baker's yeast induced fever in rabbits.

In the present study, administration of baker's yeast in rabbits caused significant elevation of temperature in 4 h. Our results matched to earlier studies reporting fever induced by baker's yeast in rats and rabbits [26,30,39]. In the classical model of fever pathogenesis, the release of pyrogenic cytokines such as interleukin IL-1, IL-6, TNF-α and interferons into the blood, induced fever [40]. *S. cerevisiae* and constituents of its cell wall, such as mannans, induce fever that is associated with increased plasma levels of IL-1b, interferon-c and TNF-α [24,25]. These mediators act on *Organum vasculosum lamina terminalis* in central nervous system (CNS). They induce prostaglandin synthesis which is the primary mediator of fever [41].

Antipyretics & non-steroidal anti-inflammatory drugs (NSAIDS) reduce fever due to their anti-inflammatory action [42]. In the present study, orally administered paracetamol (150 mg/kg) significantly decreased baker's yeast-induced fever in rabbits. It has action on a specific cyclooxygenase (COX) isoenzyme in the CNS [43]. Results are in accordance with other study results [28,39,44].

In the current study, *N. vomica* 200C and 1M normalized temperature more significantly as compared to paracetamol. *Belladonna* 200C and *Calc. phos* 200C can't reduce temperature significantly. *N. vomica* is not a routine remedy in fever. But it treats gastric fever due to excessive indulgence in drugs, alcohol and sex [9]. Mother tincture and medium to high dilutions of *N. vomica* showed antidotal effects against alcohol intoxication [10–13,45]. It might be proposed that *N. vomica* effectiveness in current study against baker's yeast induced fever was due to its antidotal property. It is prescribed after heavy doses of a substance, drug or food as it brings equilibrium, and counteracts the harmful effects of the aforementioned substances [9]. Moreover, *N. vomica* is a more suitable medicine in baker's yeast induced fever due to its similarity in gastric symptoms. Hahnemann, the founder of Homeopathy, also said in Organon of medicine (one of the original works of Hahnemann published over 250 years ago) that the primary method of treatment is to remove the fundamental cause of the disease [46]. The study also provided a preliminary guide to use *N. vomica* in gastric type of fevers in clinical practice; however, it should be confirmed in randomized clinical trials in humans with similar fever type.

According to classical Homeopathy, temperature reduction effect could not reflect the full potential for homoeopathic treatment of fever. A homeopathic medicine is selected on the basis of the global pathophysiological characteristics of the individual [47]. In the current study, two rabbits in the negative control group, two rabbits in *Belladonna* group, three in *Calc. phos* group, two rabbits in paracetamol group also developed diarrhea along with fever. Baker's yeast is also a dietary antigen that can cause diarrhea [23]. Rabbits affected with diarrhea were given metronidazole (Flagyl syrup) to control it [48]. Paracetamol reduced temperature effectively but for loose stools, this group required additional treatment of metronidazole. However, *N. vomica* group showed complete remission of suffering. This group had immediate and long term temperature reduction after receiving medicine. The stronger antipyretic effects of *Nux v* in baker's yeast induced fever may lie in the fact that it showed the closest similarity with fever type.

According to classical homoeopathy, similar remedies show more reaction (a slight aggravation before relief) (aphorisms 154, 155) [46]. The use of minimum dose may be responsible for absence of possible medicinal aggravation in current study despite the closest similarity between medicine and disease picture.

The potency should be selected according to disease energy activity (intensity), intense diseases need higher and mild diseases

require lower or medium potency [49]. Immediate and long-term temperature reduction by 200C and 1M supported that the selected potencies might be similar to disease intensity. However, antipyretic effects of lower and medium potencies of *N. vomica* should be evaluated by using same procedure first in baker's yeast induced fever in experimental animals and later in randomized clinical trials in humans (gastric fevers).

It is supposed that homeopathic medicines regulate inflammatory pathological changes by regulating natural healing dynamics (so called Hahnemann's "life force") [47]. If highly diluted homoeopathic medicines act through an influence on natural healing dynamics of the whole treated subject, at least theoretically, this action could be very sensitive to least change into experimental conditions [50]. Homeopathic medicines in ultra-high dilutions are safe and have no adverse effects [51]. Large doses of ultra-high dilutions ingestion showed no change; they do not have the power to cause adverse effects as the conventional drugs [52].

The specific effects of homeopathic medicines are of a non-molecular origin, yet provide powerful biological activities that are clinically effective [53]. It has been assumed that highly diluted substances transfer biological activity to cells by electromagnetic fields. Another working hypothesis about homeopathic ultra-high dilutions is interactions between the radiation fields of a charged molecule. The electric dipoles of water generate permanent polarization of water which becomes coherent. The specific information reaches to cell receptors like a laser [53]. A magnetic resonance imaging study on various serial dilutions showed that vigorous shaking or succession continuously alter the hydroxyl groups in the solvent of solution as dilutions become higher [54,55]. Nano bubbles (NBs) are present in homeopathic dilutions. These NBs have superstructures of increasing size and create superstructures related to specific solute [56]. However, the precise mechanism of action of these ultra-high dilutions is not known. The effects of *N. vomica* ultra-high dilutions should be checked on pyrogenic cytokines to appreciate their effect on pyrogenic cytokines during fever. Moreover, *N. vomica* ultra-high dilutions should be evaluated against other fever types as *E. coli* induced fever and stress induced hyperthermia. It would be helpful to clear the criteria of *similimum* in Homeopathy, if *N. vomica* proves ineffective against other fever types.

5. Conclusion

From the present study, it is indicative that ultra-high dilution of *N. vomica* could be an effective remedy against Baker's yeast induced fever in rabbits. *Belladonna* and *Calc. phos* ultra high dilutions showed no effect on baker's yeast induced fever**.** This study would encourage clinical use of ultra-high dilutions of *N. vomica* in similar fever type. Although statistical interferences were applied, the sample size was small to make a final conclusion. So, before clear conclusions can be made, these results should be replicated in bigger samples.

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Conflict of interest

None.

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