Circadian aspects of hyperthermia in mice induced by *Aconitum napellus*

Salvador Sánchez de la Peña, Robert B. Sothern[1], Fernando Santillán López, Irene Mendoza Lujambio[2], José Waizel-Bucay[3], Carolina Olarte Sánchez, Claudia Pérez Monroy, Eduardo Tena Betancourt[4]

Chronomics Research Center at Sección de Estudios de Posgrado e Investigación (SEPI)-Escuela Nacional de Medicina y Homeopatía (ENMyH), Instituto Politécnico Nacional (IPN), [2]Genética Molecular-Escuela Superior de Medicina-IPN, [3]SEPI-ENMyH-IPN, and [4]Bioterio Central-Centro Médico Nacional, Mexico City, México, [1]The Rhythmometry Laboratory, Department of Plant Biology, College of Biological Sciences, University of Minnesota, St. Paul, MN, USA

Submitted: 18-07-2010 Revised: 18-09-2010 Published: 25-08-2011

ABSTRACT

Background: *Aconitum napellus* (Acn) is used topically to relieve pain, itching and inflammation, and internally to reduce febrile states, among others. Any circadian time-related consequences of *Acn* administration are unknown. The objective of this study was to explore the effects of two doses of *Acn* on body temperature (BT) of mice treated at six different times over 24 hours. **Materials and Methods:** BALB/c female mice were housed in six chambers (six mice each) with air temperature 24 ± 3°C, humidity 60 ± 4%, and a 12-hours light (L)/12-hours dark cycle, but with L-onset staggered by 4 hours between chambers so that study at one external test time resulted in six test times (02, 06, 10, 14, 18 and 22 hours [h] after light onset). Rectal temperature (RT; in °C) was measured at baseline (B) and 1 hour after oral treatment with placebo (P) or two doses of *Acn* (6C and 30C, two studies each) in six studies over an 8 day span. The difference in RT for each mouse from the respective B + P timepoint mean RT was computed following each *Acn* treatment, and data from each of the six studies (original RT and difference from B + P) were analyzed for time-effect by analysis of variance (ANOVA) and for circadian rhythm by 24-hour cosine fitting. **Results:** A circadian rhythm in RT was found at B and after P (mean: 35.58°C vs. 35.69°C; peak: 15:31 h vs. 15:40 h) and after each *Acn* dose (30C or 6C). *Acn* induced hyperthermia and the overall change in BT was rhythmically significant for each dose (mean = +1.95°C vs. +1.70°C), with greatest hyperthermia observed during the L-span for each dose (peak = 08:56 h vs. 05:17 h). **Conclusion:** *Acn* administered around the clock induced hyperthermia overall and in a time-dependent manner, with greatest effects during the resting (L) span. Thus, time of day may significantly impact the outcome of *Acn* and other homeopathic treatments and should be considered in determining optimal dosing and treatment time(s) in order to increase the desired outcome and decrease undesired effects.

Key words: Aconite, *Aconitum napellus*, chronotherapy, circadian, homeopathy, hyperthermia, pharmacognosy

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.84238

Quick Response Code:

INTRODUCTION

Aconitum napellus, commonly called Aconite (*Acn*), is a perennial species of the Ranunculaceae plant family that is found in wet, shady places in hilly districts at high altitudes throughout the Northern Hemisphere mountainous regions in Europe, Asia and in northwestern North America. Its name is derived from *Aconis*, a Black Sea port in the ancient region of Bithynia (in Asia Minor corresponding roughly to modern central-northern Turkey), and *napus,* a turnip, due to the shape of its roots, which have occasionally been mistaken for horseradish. It has many English common names, such as: aconite, bear's foot, blue rocket, devil's helmet, friar's cap, helmet flower, monkshood, monksblood, queen's fettle, soldier's cap, Turk's cap and wolfsbane. *Acn* has been introduced as an ornamental garden plant and is used in herbal medicine, but it is considered a vertebrate poison (from cardio and respiratory actions) and is one of the most, if not the most, poisonous known alkaloids. Handling the plant can cause allergic reactions and rashes (even slight contact with the flowers can cause fingers to become numb)

Address for correspondence:
Dr. Salvador Sánchez de la Peña, Chronomics Research Center at Sección de Estudios de Posgrado e Investigación, ENMyH-IPN Guillermo Massieu Helguera No. 239 Ticomán, CP 07320, México City, Mexico. E-mail: ssalvadoral@gmail.com

and is dangerous if exposed to open cuts, scratches or sores or ingested.[1,2] Its use dates to remote times when Asian warriors applied *Aconitum* tincture to arrow tips to turn them into lethal weapons of war.[3] *Acn*'s poisonous properties have also been used in hunting and fishing, as well as for criminal purposes.[4]

Acn is safe only in extremely minute topical or oral doses. In ancient times, the dried tuberous root of *A. napellus* was used as a sedative medicine and painkiller. In the 2nd century BC, the Greek physician and poet Nicander of Colophon described in his "*Alexipharmaca*" the symptoms and uses of the herbal drug aconite.[5] *Aconitum* species have been used in China as an essential drug in traditional Chinese medicine for more than 2000 years,[6] and in the 2nd century AD, a famous Chinese surgeon, Hwa Tuo, employed aconite as part of a special powder in surgeries.[7] It was known to be used in the treatment of rheumatism, sciatica, and tumors, as well as a sudorific substance, but it was not until Samuel Hahnemann's studies early in the 19th century that its properties were really understood. Aconite is more closely associated with the rise and progress of homeopathy as a safe alternative to bleeding and purging than any other member of the *Materia Medica*, but it fell into disrepute until about the middle of the 20th century, when it was employed by Stoerck and given a place in the *Pharmacopoeia*.[8] In 1805, Hahnemann (1755–1843) published his studies about 27 medicines, including aconite, in a two-volume work entitled: "*Fragmenta de Viribus Medicamentorum Positivus*".[9-11] Table 1 in Hahnemann's treatise illustrates the main compounds of *Acn,* including aconitine. *Acn's* most active and toxic principle chemical ($C_{34}H_{47}N0_{11}$), in addition to less toxic mesaconitine and hypaconitine, are characterized as steroidal (diterpenoid) alkaloid compounds. Hahnemann and his therapeutic approach transformed this toxic substance into a useful anesthetic and antipyretic compound, used mainly to treat fevers, cardiac conditions and neuralgia.[9]

Acn is widely used in China, Korea, Japan, India and is gaining acceptance in Europe[4,12] as a quick and short-acting remedy in homeopathic medicine as a topical anesthetic agent to relieve pain, itching and inflammation, and as an internal agent to relieve neuralgic pain and, most commonly, to reduce febrile states associated with colds, pneumonia, laryngitis, croup, and asthma. In conjunction with conventional medicine, it has been reported that 1 day after treatment with *Acn* 200C, there was a significant reduction in postoperative pain and agitation.[13] In homeopathic therapeutics, *Acn* is often employed to treat early stages of fever in patients, including children, where it has been employed for control of fever in upper tract infections (URTIs).[14] It has also been demonstrated that there was no difference between response rates for homeopathic remedies (including *Acn)* versus conventional treatments for acute respiratory and ear complaints in an international multicenter study (57 primary care practices in 8 countries) after 14 days of therapy.[15] Other observational studies on the comparability of homeopathic treatment and conventional treatment of URTIs have also shown positive outcomes for homeopathy.[16,17] A double-blind crossover study in 27 healthy volunteers found a difference in reported responses (short-term signs and symptoms) when treated for 3 days with *Acn* 30 C versus placebo, indicating that *Acn* at that dose had a recognizable effect.[18]

Systemic effects of *Acn* follow within half an hour after its administration and seldom last over 3 hours due to a short duration of action brought about by rapid oxidization. The mode of action of *Acn* (and related alkaloids) is thought to be due to a depression of the vasomotor center and the cardiotoxicity and neurotoxicity that results from actions on the voltage-sensitive Na^+ channels of the cell membranes of excitable tissues, including the myocardium, nerves, and muscles.[1] Symptoms of *Acn* poisoning can include systemic paralysis, nausea, vomiting, dizziness, palpitations, hypotension, arrhythmia, shock, coma and death.[12] Body temperature (BT) is also lowered, probably by an initial increase in heat dissipation from gastric warmth and a general flush on the body's surface following non-lethal doses.[19] Any time of day related (i.e., circadian) consequences of *Acn* administration on these effects due to the phenomenon of "chronopharmacology" are unknown.

For centuries, traditional Chinese medicine has incorporated the concept of timing into treatment for a wide variety of ailments,[20] whereby the time and site of a treatment by acupuncture or moxibustion (heat) or a dose of herbs and other medications will differ depending on the natural cycles of the patient, which may involve the time of day, day of the week, day within the menstrual cycle, phase of the moon, and/or season. In the field of biological rhythms (chronobiology), it is now clear that administration of most therapies will result in varying positive or negative effects, depending upon differences in pharmacokinetics of drug disposition resulting from the time of day (i.e., stage of rhythm) of treatment. Therefore, information about biological rhythms can be used to maximize positive and cost-effective outcomes of various interventions. This has far-reaching implications for selecting the best timing of procedures and medications for a wide range of conditions and diseases.[21]

Attempts to time treatment according to biological rhythms in order to achieve the goal of *maximizing* the desired effect and *minimizing* undesired effects is known as "chronotherapy". Chronotherapy includes the best timing of drug treatments, medical and surgical procedures, as

well as performance and exercise scheduling. The concept of "chronopharmacology" encompasses the time of drug administration and the body's response according to the underlying temporal structure of the organism receiving it. The concept of "chronopharmacokinetics" adds "time of day" as a variable that influences the pharmacokinetics of a drug. This includes rhythmic changes in drug disposition (absorption, distribution, metabolism, elimination) that result from an interaction in processes at the molecular and membrane levels (pharmacokinetics) and rhythms in the desired and undesired effects (susceptibility).[22-24] Drugs with rhythm-dependent effects include analgesics, anticoagulants, corticosteroids, melatonin, psychobiotics, and anti-hypertensive, anti-ulcer and anti-cancer medications.[22,24,25] Many drugs have been shown to produce less toxicity, better disease control and more cures at some times of the day than others.[26-34] All of these timing concepts most certainly apply to phytomedicine and homeopathy as well.

In order to study the effects of *Acn* on BT at different times of the day, we studied the rectal temperature of female mice before and after treatment with low and high doses of *Acn* every 4 hours for 24 hours.

MATERIALS AND METHODS

Animals

In order to study the circadian time-dependent effects of *Acn*, 36 female BALB/c mice, 10 weeks of age, were obtained from the animal facilities of CINVESTAV–IPN. Six mice were housed in each of six different chambers (three/cage in two plastic cages/chamber) with environmental conditions of 24 ± 3°C and 60 ± 4% humidity with alternating changes of 12-hour light (L) and 12-hour darkness (D). Onset of L was staggered by 4 hours between the six chambers so that after synchronization to the respective LD schedules, study at only one external test time during convenient working hours allowed six different circadian stages (times) to be tested concomitantly (02, 06, 10, 14, 18 and 22 hours after light onset: HALO).[35] This was possible since the local LD schedule synchronizes the body's endogenous biological clock to a 24-hour schedule, thereby setting the peak and trough of the endogenous circadian rhythms to specific times of the environmental day (L) and night (D) spans. Animals were fed with Purina Chow 5010 and sterilized tap water that were renewed every other day when bedding was changed (if a chamber was dark, a red dim room light was used in order to avoid any exposure of white light). These studies were approved by the local Institutional Animal Welfare Committee and were designed to meet the ethical standards of biological rhythm research.[36]

Acn decimal dilutions of Hahnemann

Highly diluted natural complexes of *Acn* are used as forms of therapy and follow Hahnemann's ancient homeopathic techniques for dilution using a centesimal or C scale that dilutes a substance by a factor of 100 at each stage.[37,38] For example, for a 2C solution, the C scale requires that a substance be diluted to 1 part in 100, and then 10% of that diluted solution diluted by a further factor of 100. This works out to one part of the original substance in 10,000 parts of the solution (10^{-6}). In our case, a "mother" tincture of *Acn* was purchased from authorized agencies (At Mexico City, Mexico) sanctioned by the Mexican Health Ministry, which assures the quality (endotoxin free) and physicochemical composition of the product. Following Mexican homeopathic regulations and starting from the original mother tincture – an ethanolic extract in this case – several dynamizations/succussion (shaking by forceful striking) and serial dilutions in distilled water were performed to obtain 6th (6C) and 30th (30C) dilutions. (Note: Acn 30C is a common dosage used in homeopathy[18] and there have been no reported adverse effects from homeopathic remedies above 12C.)[39] The final solutions contained *A. napellus*, all in decimal dilutions of Hahnemann (dH) in distilled water, were colorless and odorless, and had a 1% alcohol concentration. This complex was maintained at room temperature and vigorously shaken (succussed) immediately before each treatment. Placebo (P) consisted of only distilled water and 1% alcohol at room temperature.

Temperature measurement

Rectal temperature (RT) was obtained using an electrical tele-thermometer instrument (Yellow Spring Instrument Co., OH, USA , Model 43TA, SN93D05034) and recorded to the nearest 0.5°C. RT was measured manually for 20 seconds using a plastic probe inserted 3 mm into the rectum of each of the 36 female mice in each of the six studies, resulting in 216 total values (6/mouse).

Study design and treatments

Sampling was staggered across the six chambers over the approximate 1 hour it took to complete all procedures and results were assigned to the midpoint of the total sampling span. Thus, in each study and beginning 30 min before the targeted midpoint, mouse #1 was used from chambers 1 through 6, then the second mouse from each chamber and so on until the sixth mouse per chamber, resulting in six mice from each chamber being studied, which corresponded to one circadian sampling time (target times = 02, 06, 10, 14, 18 and 22 HALO).

After 3 weeks of synchronization to the staggered LD schedules, RT of all mice was measured at baseline or 1 hour after oral treatment with P or a dose of *Acn* (6C or 30C diluted from original tincture) in six studies: 1) baseline

(B), 2) placebo (P), 3) *Acn* 30C, 4) *Acn* 6C, 5) *Acn* 30C, and 6) *Acn* 6C. Following RT measurement at baseline, each mouse immediately received an oral dose of P and then RT was re-measured after 1 hour. RT was subsequently measured only 1 hour after the oral *Acn* treatments in the ensuing four experiments. Studies were carried out over a single 8-day span from a Monday to the next Monday in 2005 with a 24-hour span between studies 1 and 2 (Oct 24) and 3 (Oct 25), 3 and 4 (Oct 26), a 48-hour span from 4 to 5 (Oct 28), and a 72-hour span from 5 to 6 (Oct 31). Even though the systemic effects of *Acn* are short-acting, studies 5 and 6 were designed to monitor the retest effects of *Acn* after intervals longer than 24 hours between dosing.

Statistical analyses

Following placebo or *Acn* treatment, the difference in RT for each mouse from the respective timepoint baseline RT was computed. Data from each of the six studies (original RT and change/difference from baseline) were analyzed for time-effect across the six timepoints by one-way analysis of variance (ANOVA) and for circadian rhythm characteristics by the single cosinor procedure[40] by approximation of each time series data by the least-squares linear regression fit of a single component (24-hour) cosine using the Chronolab statistical package.[41] A *P*-value for the rejection of the zero-amplitude assumption was determined by an F-test of the variance accounted for by the fit of the 24-hour cosine versus the variance accounted for by a straight line approximation of the arithmetic mean. Rhythm detection and/or a time-effect by ANOVA was considered statistically significant if $P \leq 0.05$. Rhythm characteristics determined from the best-fitting cosine model include: the "mesor" (M, the middle of the cosine representing an adjusted 24-hour average, which equals the arithmetic mean if sampling is equidistant and there are no missing data or timepoints, as in our study); "amplitude" (A, half the distance from the peak and trough of the best-fitting curve, with 2A indicating the predictable range of change); and the "phase" of the cosine model (Ø, in hh:mm from an external point, such as local midnight or L-onset, as in our case), with the peak of a single component cosine called the "acrophase" (aØ, *acro* = peak). Rhythm parameters (mesor, amplitude, acrophase) between studies were compared by parameter test.[42]

RESULTS

Timepoint means ± SE for each treatment and study are listed in Table 1 and displayed in Figures 1 and 2. During baseline conditions (B), mice displayed the anticipated

Table 1: Body temperature timepoint means of mice at baseline and 1h after treatment with placebo or *Acn* at 6 circadian stages*

Study	Treatment(s)	N	Treatment time (HALO*):						
			Overall	02:00h	06:00h	10:00h	14:00h	18:00h	22:00h
			Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
	Original body temperature (°C)								
1	Baseline (B)	36	35.58 ±0.14	35.17 ±0.17	35.33 ±0.36	35.42 ±0.35	36.08 ±0.27	**36.33** ±0.36	35.17 ±0.28
2	Placebo (P)	36	35.69 ±0.16	35.25 ±0.44	35.33 ±0.36	35.50 ±0.32	36.33 ±0.38	**36.42** ±0.40	35.33 ±0.28
1+2	B+P	72	35.64 ±0.11	35.21 ±0.23	35.33 ±0.24	35.46 ±0.23	36.21 ±0.23	**36.38** ±0.25	35.25 ±0.19
3	*Acn* 30C	36	37.26 ±0.16	36.42 ±0.37	36.83 ±0.46	**38.17** ±0.17	37.33 ±0.17	38.08 ±0.08	36.75 ±0.38
4	*Acn* 6C	36	37.26 ±0.12	37.42 ±0.27	37.50 ±0.34	**37.67** ±0.31	37.42 ±0.27	37.00 ±0.22	36.58 ±0.15
5	*Acn* 30C	36	37.92 ±0.13	37.92 ±0.24	37.58 ±0.30	38.58 ±0.20	**38.67** ±0.11	37.83 ±0.17	36.92 ±0.30
6	*Acn* 6C	36	37.42 ±0.13	**38.17** ±0.11	37.33 ±0.36	37.92 ±0.35	37.33 ±0.21	36.83 ±0.21	36.92 ±0.27
3+5	*Acn* 30C	72	37.59 ±0.11	37.17 ±0.31	37.21 ±0.29	**38.38** ±0.14	38.00 ±0.22	37.96 ±0.10	36.83 ±0.23
4+6	*Acn* 6C	72	37.34 ±0.09	**37.79** ±0.18	37.42 ±0.24	**37.79** ±0.23	37.38 ±0.16	36.92 ±0.15	36.75 ±0.16
	Change in body temperature (°C) from Controls (P from B or Acn from B+P)								
2	P	36	0.11 ±0.10	0.08 ±0.33	0.00 ±0.00	0.08 ±0.40	**0.25** ±0.21	0.08 ±0.15	0.17 ±0.25
3	*Acn* 30C	36	1.63 ±0.18	1.21 ±0.42	1.50 ±0.61	**2.71** ±0.34	1.13 ±0.25	1.71 ±0.43	1.50 ±0.41
4	*Acn* 6C	36	1.63 ±0.19	**2.21** ±0.40	**2.17** ±0.60	**2.21** ±0.39	1.21 ±0.43	0.63 ±0.36	1.33 ±0.36
5	*Acn* 30C	36	2.28 ±0.17	2.71 ±0.45	2.25 ±0.38	**3.13** ±0.28	2.46 ±0.35	1.46 ±0.45	1.67 ±0.33
6	*Acn* 6C	36	1.78 ±0.20	**2.96** ±0.21	2.00 ±0.47	2.46 ±0.33	1.13 ±0.50	0.46 ±0.49	1.67 ±0.26
3+5	*Acn* 30C	72	1.95 ±0.13	1.96 ±0.37	1.88 ±0.36	**2.92** ±0.22	1.79 ±0.29	1.58 ±0.30	1.58 ±0.25
4+6	*Acn* 6C	72	1.70 ±0.14	**2.58** ±0.24	2.08 ±0.36	2.33 ±0.25	1.17 ±0.31	0.54 ±0.29	1.50 ±0.22

*At each of 6 times over 24h in LD 12:12 (02, 06, 10, 14, 18 & 22 Hours After Lights-On [HALO]), 6 Balb/c female mice (36 total) received an oral dose of placebo or *Aconitum napellus (Acn)* in high dilution (30C) or low dilution (6C) dissolved in distilled water. Rectal temperature (°C) was measured in each mouse one hour after each dose. The same mice were used for each study. Baseline temp obtained 5 minutes prior to placebo treatment. Baseline & placebo data combined for *Acn* comparisons. Maximum temp in **bold**, minimum underlined (see Table 2 for results from analyses for circadian time-effects)

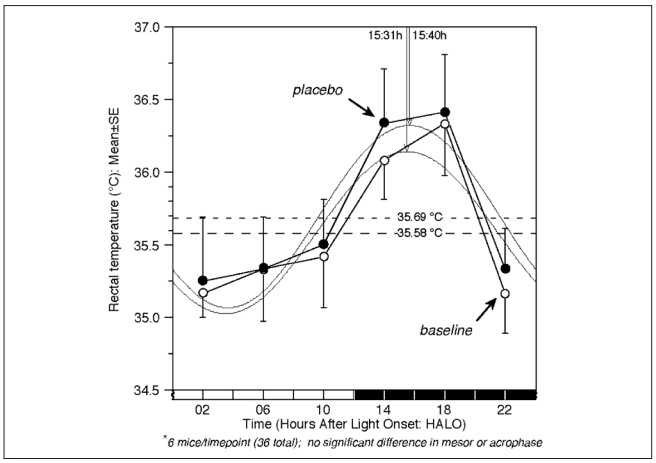

Figure 1: Chronograms showing circadian patterns for mouse body temperature at baseline (B) and 1 hour later after oral placebo (P) at six treatment times [in hours after lights on (HALO, h)]. 4-hour means ± SE from mice studied in LD 12:12 (6 mice/timepoint, 36 total) are shown with best-fitting 24-hour cosine. For time-effect, *P*-values from fit of a 24-hour cosine were 0.013 for B and 0.017 for P. No significant differences between B versus P were found for 24-hour mean (mesor) (35.58 vs. 35.69°C), amplitude (0.56 vs. 0.63°C), or acrophase (15:31 h vs. 15:40 h), allowing averaging for further comparison following *Acn* treatments. Dark bar = 12-hour dark/activity span

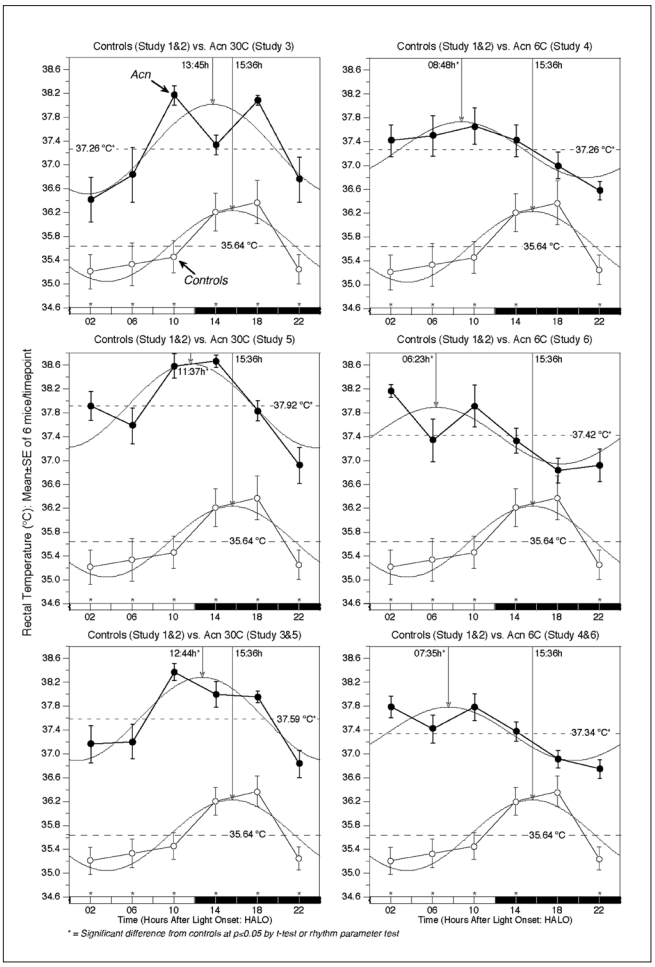

Figure 2: Chronograms showing circadian patterns for mouse body temperature at baseline (B + P) and 1 hour after oral *Acn* (30C or 6C) at six treatment times [in hours after lights on (HALO)]. 4-hour means ± SE from mice studied in LD 12:12 (6 mice/timepoint, 36 total) are shown with best-fitting 24-hour cosine. For time-effect, the fit of 24-hour cosine was significant at $P < 0.05$ for each series [see Table 2 for specifics]. An overall hyperthermia was significant following each *Acn* treatment in studies 3–6 separately or when the two studies of each dose were combined when compared with controls, while calculated acrophases (peaks) were significantly advanced, except for Study 3 (*Acn* 30C). Dark bar = 12-hour dark/activity span

circadian variation in their BT, with the highest values found in the middle of the dark/activity span and the lowest values found during the light/resting span [Figure 1]. A significant rhythm in RT was found at B and after P, with no significant differences in rhythm parameters (M = 35.58° vs. 35.69°, Ø = 15:31 hours vs. 15:40 hours) [top rows in Table 2]. Overall timepoint means for B and P values were thus computed to serve as the baseline values for comparison with each *Acn* series.

A significant rhythm was found in original RT after all *Acn* doses in studies 3–6 and overall for 30C in studies 3 and 5 and for 6C in studies 4 and 6, but with highest values shifted to late-L and early-D for 30C and during the L-span for 6C [middle rows in Tables 1 and 2; Figure 2]. *Acn* induced significant hyperthermia overall compared with B + P after both doses (M = 37.59° for 30C vs. 37.34° for 6C), and at each timepoint, except for 6C at 18 HALO, which increased but not significantly [Figure 2]. The RT Øs following *Acn* treatment (12:44 hours for 30C vs. 07:35 hours for 6C) were significantly advanced from baseline (15:36 hours) by ~3 hours and 8 hours, respectively, and different from each other by ~5 hours [Table 2; Figure 2].

Timepoint means ± SE for change in RT from baseline following P or *Acn* treatment in studies 3–6 and combined for 30C from studies 3 and 5 and for 6C from studies 4 and 6 are listed in Table 1 (bottom rows) and changes after *Acn* are displayed in Figure 3. There were only minor, but nonsignificant changes in baseline RT following P that ranged from 0.0 to +0.25°C [Table 1] and were not rhythmic [Table 2]. The change in BT from baseline was rhythmically significant in studies 4–6 and for each *Acn* dose overall when the comparable two studies were combined. There was a significant difference between overall means in study 5 carried out after a 48-hour break (*Acn* 30C, +2.28°) and study 6 carried out after a 72-hour break (*Acn* 6C, +1.78°) in treatments, but these levels were not significantly different between studies 3 and 4 (+1.62° for 30C vs. +1.63° for 6C) or when both studies for each dose were combined (M = +1.95° for 30C vs. +1.70° for 6C). Overall, the least hyperthermia was observed at 18 HALO following 6C treatment (+1.17°), which was significantly different from 30C (+1.79°) at the same time. Greatest

Table 2: Statistical evaluation of circadian stage-dependent variations in body temperature of mice treated with *Acn

			Analysis for time-effect:**							
			ANOVA		24h Cosine					
Study	Treatment(s)	N	F	p	p	Mesor ± SE	Amp ± SE	(2A)	aØ	(95% Limits)
	Original body temperature (°C)									
1	Baseline (B)	36	2.7	0.040	0.013	35.58 ±0.13	0.56 ±0.18	(1.12)	15:31h	(12:52, 18:12h)
2	Placebo (P)	36	2.1	0.089	0.017	35.69 ±0.15	0.63 ±0.21	(1.26)	15:40h	(12:52, 18:28h)
1+2	B+P	72	5.1	<0.001	<0.001	35.64 ±0.09	0.59 ±0.13	(1.18)	15:36h	(13:48, 17:24h)
	Original body temperature (°C)									
3	*Acn* 30C	36	5.7	<0.001	0.002	**37.26** ±0.14	0.75 ±0.19	(1.50)	13:45h	(11:36, 15:52h)
4	*Acn* 6C	36	2.2	0.079	0.016	**37.26** ±0.11	0.47 ±0.15	(0.94)	**08:48h**	(06:00, 11:36h)
5	*Acn* 30C	36	8.1	<0.001	<0.001	**37.92** ±0.11	0.70 ±0.15	(1.40)	**11:37h**	(09:52, 13:20h)
6	*Acn* 6C	36	4.0	0.007	0.030	**37.42** ±0.12	0.47 ±0.17	(0.94)	**06:23h**	(03:16, 09:32h)
3+5	*Acn* 30C	72	7.0	<0.001	<0.001	***37.59*** ±0.10	0.70 ±0.13	(1.40)	***12:44h***	(11:12, 14:16h)
4+6	*Acn* 6C	72	2.7	0.028	<0.001	***37.34*** ±0.08	0.45 ±0.11	(0.90)	***07:35h***	(05:32, 09:36h)
	Change in body temperature (°C) from Controls (P from B or Acn from B+P)									
2	P	36	0.1	0.989	0.880	0.12 ±0.10	0.07 ±0.14	(0.14)	16:42h	- -
3	*Acn* 30C	36	1.8	0.135	0.396	1.62 ±0.18	0.36 ±0.26	(0.72)	10:23h	- -
4	*Acn* 6C	36	2.4	0.060	0.007	1.63 ±0.17	0.83 ±0.24	(1.66)	05:50h	(03:24, 08:16h)
5	*Acn* 30C	36	2.7	0.037	0.025	*2.28* ±0.16	0.65 ±0.23	(1.30)	08:10h	(05:12, 11:08h)
6	*Acn* 6C	36	5.3	0.001	<0.001	*1.78* ±0.17	1.00 ±0.24	(2.00)	04:50h	(02:52, 06:48h)
3+5	*Acn* 30C	72	5.3	<0.001	0.030	1.95 ±0.13	0.48 ±0.18	(0.96)	*08:56h*	(05:48, 12:04h)
4+6	*Acn* 6C	72	7.4	<0.001	<0.001	1.70 ±0.12	0.90 ±0.17	(1.80)	*05:17h*	(03:52, 06:44h)

*At each of 6 times over 24h in LD 12:12 (02, 06, 10, 14, 18 & 22 Hours After Lights-On [HALO]), 6 Balb/c female mice (36 total) received an oral dose of placebo or *Aconitum napellus (Acn)* in high dilution (30C) or low dilution (6C) dissolved in distilled water. Rectal temperature (°C) was measured in each mouse one hour after each dose. Baseline temp obtained 5 minutes prior to placebo treatment. The same mice were used for each study. **Analyses for time-effect: ANOVA = analysis of variance across timepoint means using all values per treatment(s). Cosinor = least-squares fit of 24h cosine to all data (in °C). Mesor = rhythm-adjusted overall 24h mean, Amp (A) = amplitude (2A = predictable peak-trough range of cosine); aØ (acrophase) = peak of cosine in hr:min from L-onset; 95% limits listed if p≤0.05). Significant difference from controls in **bold,** or between *Acn* doses in *italics* if parameter test *P*≤0.05.

hyperthermia was observed during the L-span for each dose (Ø = 08:56 hours for 30C vs. 05:17 hours for 6C) [bottom rows in Table 2; Figure 3]; these Øs were also significantly different from each other by about 3.75 hours by parameter test.

DISCUSSION

For centuries, *Acn* has been used as a poison capable of inducing death – the ancient Greeks called it the Queen of Poisons, the deadliest of all. Single oral doses as low as 1–2 mg have been reported to be lethal in humans.[43-45] Eventually, it was identified as a neurotoxic compound that acts upon nerve hypothalamic centers, as well as on sympathetic peripheral nerves.[12] The cardiotoxicity and neurotoxicity of *Acn* and related alkaloids result in a combination of neurological, cardiovascular and gastrointestinal symptoms, and in high doses can induce paralysis and death.[1] The precise neurophysiologic mechanism of *Acn* action is not completely known, but its main and most toxic component, aconitine, which is characterized by a steroidal alkaloid chemical structure, has been studied in the central nervous system of rats at the level of the hippocampus neuronal activity.[46] Specialized neurophysiological research, based on patch-clamp techniques mainly related to the neuron excitability process, has demonstrated that a low physiological *Acn* dose (10^{-6} M) affects individual pre-synaptic rat neuron excitability by opening Na+ channels.[47]

Used as a homeopathic agent at very low doses (e.g., 6C and 30C), *Acn* has shown analgesic and anti-inflammatory properties. According to homeopathic theory, it was expected that low doses of *Acn* would induce hypothermia in the BT of female mice. However, when we administered two different oral doses of *Acn* to mice at six times around the clock on four occasions (each dose studied twice), the opposite effect was observed 60 min after treatment: each dose induced *hyper*thermia overall, but in a rhythmic, time-dependent manner, with the greatest effects during the resting (L) span after 30C and late-L to early-D (dark/activity) span after 6C [Figure 2]. This is not inconsistent, however, with the *Eclectic Materia Medica* description of an ultimate lowering of body temperature following an increase in heat dissipation from gastric warmth and a flush on the body's surface shortly after *Acn* treatment.[19]

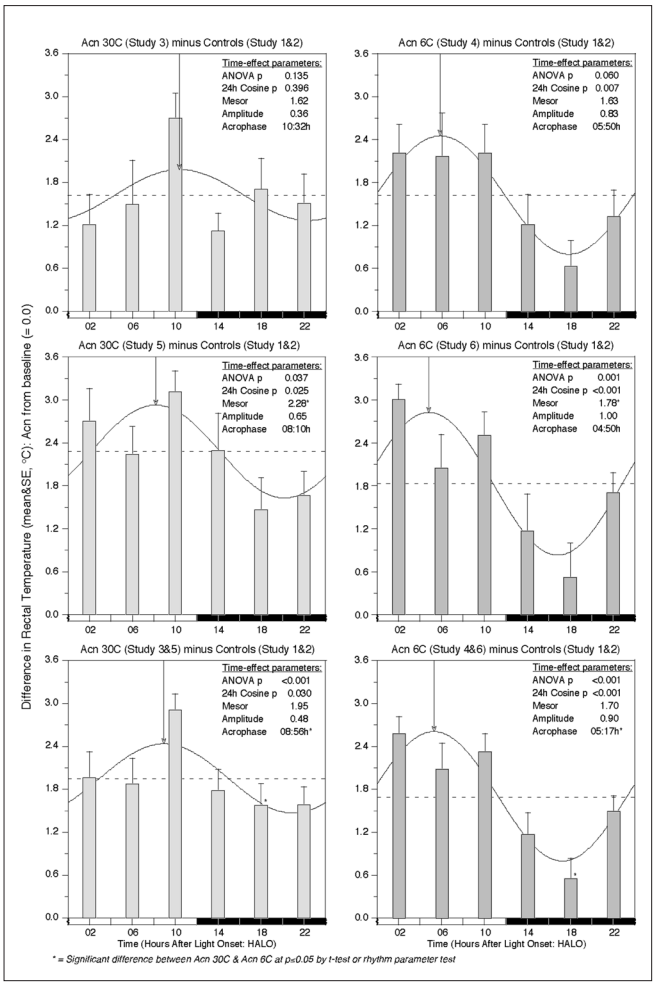

Figure 3: Bar graphs indicating a hyperthermic change from baseline following *Acn* treatments (30C and 6C) at six different times in four studies and when the two studies of each dose were combined. At each timepoint, body temperature was significantly increased over control values except at 18 HALO for *Acn* 6C [see Figure 2]. While hyperthermia was noted at each timepoint and after each dose level, maximum hyperthermia was noted during the L-span (resting) for each *Acn* dose level, while the smallest hyperthermic effects were noted during the D-span (activity) in these nocturnally active mice

Also, from an allopathic point of view, one would expect that the extremely low dose of *Acn* 30C would not have any effect on mouse BT, but the hyperthermia that was observed was more or less comparable to that noted when mice were treated with the higher dose of *Acn* 6C (overall RT changes: 30C, +1.95 ± 0.13° vs. 6C, +1.70 ± 0.14°, n.s.) [Table 2].

The oral gavage procedure itself could not be responsible for the hyperthermia observed since the delivery of placebo (i.e., water and 1% ethanol) by the same method induced modest, but nonsignificant increases in RT from baseline overall (means: B, 35.58 ± 0.14° vs. P, 35.69 ± 0.16°, n.s.) [Table 1] and at some timepoints and not others (e.g., +0.0° at 06 HALO vs. +0.25° at 14 HALO) [Table 2]. No significant time-effects or 24-hour rhythm was found in the B to P changes, which were all substantially smaller than those induced by either of the *Acn* doses at the same circadian timepoints. Moreover, there were no significant differences between the rhythmic temperature parameters (M, A, Ø) in baseline versus placebo-treated mice [Table 2]. Therefore, we chose to incorporate the minor P-effects (possibly due to handling and/or the fact that RT after P was measured 1 hour after B measurements and minor changes could be due to the time course of the underlying circadian rhythm in BT) by averaging the B + P data for use as baseline RT data when computing changes from RT following *Acn* dosings.

Any influence on overall BT changes due to the 4–5 day estrus cycle of female mice were thought to be minimal in affecting the BT response to *Acn* dosing since the mice were dispersed over six separate boxes and would not be socially synchronized as a group by smell or sound. Nevertheless, it would be of interest in any future study to test male mice, as well as measure RT before as well as after 1 hour following each *Acn* treatment on each study day in order to obtain separate study baseline values (that may reflect effects of repeated handling and/or daily changes in BT due to estrus cycle changes), rather than rely on only the temperature values obtained at the beginning of the entire study prior to any treatment.

In order to study the chronic physiologic effects of administration on body weight (BW) and RT changes in mice, a recent investigation treated male mice intragastrically daily with *Acn* (1 mg/kg/day) for 22 days and measured BW and RT on days 0, 1, 3, and every 4 days thereafter to day 22.[48] *Acn*-treated mice showed virtually no BW gain over the 22-day study when compared with placebo-treated controls and day 0. However, when RT was measured at 10-min intervals for 90 min following each *Acn* treatment, a transient hypothermia was noted to occur within the first 30 min of dosing, followed by a gradual increase to the end of the 90 min observation span. The extent and duration of hypothermia lessened throughout the study such that on study day 11 and thereafter, RT had always returned to its pretreatment level or higher by 60–90 min. The authors concluded that long-term administration of *Acn* suppresses hypothermia and ultimately warms the body.

Another recent study of male mice exposed to room temperature of 4°C for 10 days to induce chronic cold stress reached the same conclusion when BW and RT were measured every 2 days.[49] Core BT in untreated mice was significantly decreased by about 1°C by day 5 of the cold exposure when compared with mice maintained at normal room temperature (24°C). However, in mice receiving *Acn*

added to a standard murine powdered chow available *ad libitum*, the BT reduction was significantly counteracted in a dose-dependent manner (0.063, 0.25 and 1.0 g/kg/day were tested) such that there was no significant difference for any dose on any treatment day from the normal mice (the *Acn* 1.0 g dose actually showed significantly higher BT than normal controls on days 5, 7 and 9 after cold initiation). BW gain tended to be lower in the *Acn*-treated mice compared with room temperature controls, but there was no difference between cold-stressed controls and *Acn*-treated mice. Since the *Acn* treatment did not increase core BT in mice under normal room temperature conditions, the authors concluded that *Acn* did not directly stimulate thermogenesis, but rather facilitated a non-shivering physiological thermoregulation that occurs in brown adipose tissue, wherein the heat is produced through the metabolism of free fatty acids in the mitochondria.

Of note, the procedures in the two studies mentioned above appear to have been undertaken only once daily at a time(s) which may have been convenient to the researchers. In the study by Wada *et al*.,[48] there is no mention of the LD schedule for the mice or the time of day of *Acn* dosing and RT measurements. Assuming that the mice were kept in dark at night and with lights on during the day, the study was most likely carried out in the morning (e.g., between 08:00 and 12:00 hours or 02–06 HALO). In the study by Makino *et al*.,[49] they reported that the mice were housed under a 12-hour L–12 hour D schedule with L-on from 07:00 to 19:00 hours and BT was measured between 13:00 and 15:00 hours, which would be in the middle of the daily resting span (06–09 HALO). Both of these studies were thus carried out at only one of the six different circadian times that we used in our study in order to consider the well-known circadian variation in mouse body temperature (i.e., BT reaches its minima during mid-L and maxima during mid-D). Thus, for proper comparison to the human sleep–wake schedule, an extrapolation of the 22-day study mouse protocol of Wada and the 10-day study protocol of Makino would require similar treatment(s) during rest/sleep (i.e., at night after sleep onset).

CONCLUSION

Acn administered in two different doses (6C and 30C) to healthy mice at six times 4 hours apart over 24 hours each induced hyperthermia overall and in a significant time-dependent (i.e., circadian) manner, with greater effects during the resting (L) span in nocturnally active mice. These results suggest that time of day may significantly impact the outcome of not only *Acn,* but also other homeopathic treatments used in the field of pharmacognosy. A chronobiologic approach that considers timing presents a new perspective for exploring the temporal mechanisms of action(s) by *Acn* and other homeopathic compounds in relation to mitochondrial and genetic involvement in thermal regulation at the level of hypothalamic centers, as well as their affect on neuroendocrine–immune network interactions.[50] With regard to homeopathic treatments, the concept of "chronotherapy" should be considered in determining optimal dosing and time of treatment(s) in order to increase the desired outcome and decrease the undesired effects of homeopathic procedures. At the very least, time(s) of treatment(s) should be recorded and reported for any future comparisons.

ACKNOWLEDGMENTS

Grant support of the ICyT-DF, No. PICD08-82 and IML and SIP-IPN No. 20101632 to the principal author (SSP) is acknowledged. SSP is also grateful to the fellowship programs of de Estímulos al Desarrollo de Investigación (EDI) of the Instituto Politécnico Nacional (IPN), Mexico. JWB and IML would like to acknowledge grants conferred by the Comisión de Operación y Fomento de Actividades Académicas (COFAA) and Programa de Estímulos al Desarrollo Docente del IPN, Mexico.

All plastic cages, bottles and taps were kindly supplied by Dr. Eduardo Tena, Director of Bioterio Central Facilities at the del CMN Siglo XXI. Purina Chow 5010 was kindly provided by Fundación de Investigación Crono Oncológica A.C.

REFERENCES

1. Chan TY. Aconite poisoning. Clin Toxicol (Phila) 2009;47:279-85.
2. Aconite. Review of Natural Products. Facts and Comparisons 4.0. St. Louis, MO. Wolters Kluwer Health, Inc.; July 2009. Available from: http://www.drugs.com/npc/aconite.html#ixzz118RjCfVi. [last accessed on 2010 Jul 3].
3. Bisset NG. Arrow poisons in China. Part II. Aconitum-botany, chemistry, and pharmacology. J Ethnopharmacol 1981;4: 247-336.
4. Bisset NG. One man's poison, another man's medicine? J Ethnopharmacol 1991;32:71-81.
5. Skaltsa H, Philianos S, Papaphilippou G. The Aconitine described by Nicander and today. Rev Hist Pharm (Paris) 1997;45:405-10.
6. Singhuber J, Zhu M, Prinz S, Kopp B. Aconitum in traditional Chinese medicine: A valuable drug or an unpredictable risk? J Ethnopharmacol 2009;126:18-30.
7. Chu NS. Legendary Hwa Tuo's surgery under general anesthesia in the second century China. Acta Neurol Taiwan 2004;13:211-6.
8. American Institute of Homoeopathy. The Pharmacopoeia of the United States. Falls Church, Virginia. USA. American Institute of Homoeopathy, 1979.
9. Gotfredsen E. The incomplete reference-guide to Herbal medicine. Liber Herbarum II. Available from: http://www.liberherbarum.com/kilde026.htm. [last accessed on 2010 Jul 3].
10. Clarke HJ. A Dictionary of Practical Materia Medica. Available from: http://www.farmaciasantantonio.it/Homeoint/clarke/a/acon.htm. [last accessed on 2010 Jul 3].
11. Duke AJ. Dr. Duke's Phytochemical and Ethnobotanical

Databases. Available from: http://www.ars-grin.gov/cgi-bin/duke/farmacy2.pl. [last accessed on 2010 Jul 3].

12. Ameri A. The effects of Aconitum alkaloids on the central nervous system. Prog Neurobiol 1998;56:211-35.
13. Oberbaum M, Scheiber R, Rosenthal C, Itzchaki M. Homeopathic treatment in emergency medicine: A case series. Homeopathy 2003;92:44-7.
14. Steinsbekk A, Lewith G, Fønnebø V, Bentzen N. An exploratory study of the contextual effect of homeopathic care. A randomised controlled trial of homeopathic care vs. self-prescribed homeopathic medicine in the prevention of upper respiratory tract infections in children. Prev Med 2007;45:274-9; discussion 280-1.
15. Haidvogl M, Riley DS, Heder M, Brien S, Jong M, Fischer M, *et al*. Homeopathic and conventional treatment for acute respiratory and ear complaints: A comparative study on outcome in the primary care setting. BMC Complement Altern Med 2007;7:7.
16. Trichard M, Chaufferin G, Nicoloyannis N. Pharmacoeconomic comparison between homeopathic and antibiotic treatment strategies in recurrent rhinopharyngitis in children. Homeopathy 2005;94:3-9.
17. Friese KH, Kruse F, Lüdtke R, Möller H. The homeopathic treatment of otitis media in children- comparisons with conventional therapy. Int J Clin Pharmacol Ther 1997;35:296-301.
18. Piltan D, Rist L, Simões-Wüst P, Saller R. Test of a homeopathic dilution of Aconitum napellus. A clinical, randomized, double-blind, controlled crossover study in healthy volunteers. Forsch Komplementmed 2009;16:168-73.
19. Felter HW. PART II - Individual Drugs. Aconitum Napellus. The Eclectic Materia Medica, Pharmacology and Therapeutics. Cincinnati, OH: Eclectic Medical Publications; 1922. p. 4-13.
20. Wu J. Neijing chronobiologic medical theories. Chin Med J 1982;95:569-78.
21. Sothern RB. Hours of changing resistance. In: Koukkari WL, Sothern RB, editors. Introducing Biological Rhythms. A primer on the temporal organization of life, with implications for health, society, reproduction and the natural environment. New York: Springer; 2006. p. 470-80.
22. Reinberg A, Smolensky M. Circadian changes in drug disposition in man. Clin Pharmacokinet 1982;7:401-20.
23. Ritschel WA, Forusz H. Chronopharmacology: A review of drugs studied. Methods Find Exp Clin Pharmacol 1994;16:57-75.
24. Bruguerolle B. Chronopharmacokinetics. Current status. Clin Pharmacokinet 1998;35:83-94.
25. Smolensky MH, Haus E. Circadian rhythms and clinical medicine with applications to hypertension. Am J Hypertens 2001;14:280S-90.
26. Reinberg A, Smolensky M. Biological Rhythms and Medicine. Cellular, Metabolic, Physiopathologic, and Pharmacologic Aspects. New York: Springer-Verlag; 1983. p. 305.
27. Rivard GE, Infante-Rivard C, Hoyeux C, Champagne J. Maintenance chemotherapy for childhood acute lymphoblastic leukemia: Better in the evening. Lancet 1985;2:1264-6.
28. Rivard GE, Infante-Rivard C, Dresse MF, Leclerc JM, Champagne J. Circadian time-dependent response of childhood lymphoblastic leukemia to chemotherapy: A long-term follow-up study of survival. Chronobiol Int 1993;10:201-4.
29. Lemmer B, editor. Chronopharmacology: Cellular and Biochemical Interactions. New York: Marcel Dekker; 1989. p. 720.
30. Labrecque G, Bélanger PM. Biological rhythms in the absorption, distribution, metabolism and excretion of drugs. Pharmacol Ther 1991;52:95-107.
31. Redfern PH, Lemmer B, editors. Physiology and Pharmacology of Biological Rhythms. Berlin: Springer-Verlag; 1997. p. 668.
32. Sothern RB. Examples of applied chronotherapy. In: Koukkari WL, Sothern RB, editors. Introducing Biological Rhythms. A primer on the temporal organization of life, with implications for health, society, reproduction and the natural environment. New York: Springer; 2006. p. 480-6.
33. Smolensky MH, Hermida RC, Ayala DE, Tiseo R, Portaluppi F. Administration-time-dependent effects of blood pressure-lowering medications: Basis for the chronotherapy of hypertension. Blood Press Monit 2010;15:173-80.
34. Hermida RC, Ayala DE, Mojón A, Fernández JR. Influence of circadian time of hypertension treatment on cardiovascular risk: Results of the MAPEC study. Chronobiol Int 2010;27:1629-51.
35. Sothern RB. Time of day versus internal circadian timing references. J Infus Chemother 1995;5:24-30.
36. Portaluppi F, Touitou Y, Smolensky MH. Ethical and methodological standards for laboratory and medical biological rhythm research. Chronobiol Int 2008;25:999-1016.
37. Hahnemann S. The Organon of Medicine. 6th ed. 1921. (modern English translation by Kunzli J, Naude A, Pendleton P. 1982. p. 277).
38. Cook T. Samuel Hahnemann, the Founder of Homeopathy. UK: Thorsons; 1981.
39. La Pine MP, Malcomson FN, Torrance JM, Marsh NV. Night shift: Can a homeopathic remedy alleviate shift lag? Dimens Crit Care Nurs 2006;25:130-6.
40. Nelson W, Tong YL, Lee JK, Halberg F. Methods for cosinor rhythmometry. Chronobiologia 1979;6:305-23.
41. Mojón A, Fernández JR, Hermida R. Chronolab: An interactive software package for chronobiologic time series analysis written for the Macintosh computer. Chronobiol Int 1992;9:403-12.
42. Bingham C, Arbogast B, Cornélissen GC, Lee JK, Halberg F. Inferential statistical methods for estimating and comparing cosinor parameters. Chronobiologia 1982;9:397-439.
43. Camps FE. Gradwohl's Legal Medicine. 2nd ed. Bristol: John Wright and Sons; 1968. p. 674.
44. Rentoul E, Smith H. Glatier's Medical Jurisprudence and Toxicology. Edinburgh, Scotland: Churchill Livingstone; 1973. p. 520-1.
45. Singh S, Fadnis PP, Sharma BK. Aconite poisoning. J Assoc Physicians India 1986;34:825-6.
46. Ameri A. Structure-dependent inhibitory action of the Aconitum alkaloids 14-benzoyltalitasamine and talitasamine in rat hippocampal slices. Naunyn Schmiedebergs Arch Pharmacol 1998;357:585-92.
47. Yamanaka H, Doi A, Ishibashi H, Akaike N. Aconitine facilitates spontaneous transmitter release at rat ventromedial hypothalamic neurons. Br J Pharmacol 2002;135:816-22.
48. Wada K, Nuhira M, Ohno Y. Effects of chronic administrations of aconitine on body weight and rectal temperature in mice. J Ethnopharmacol 2006;105:89-94.
49. Makino T, Kato K, Mizukami H. Processed aconite root prevents cold-stress-induced hypothermia and immuno-suppression in mice. Biol Pharm Bull 2009;32:1741-8.
50. Sánchez de la Peña S. The feedsideward of cephalo-adrenal immune interactions. Chronobiologia 1993;20:1-52.

Cite this article as: de la Peña SS, Sothern RB, López FS, Lujambio IM, Waizel-Bucay J, Sánchez CO, *et al*. Circadian aspects of hyperthermia in mice induced by *Aconitum napellus*. Phcog Mag 2011;7:234-42.

Source of Support: Nil, **Conflict of Interest:** None declared.