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# Implantable Radiotelemetry System in Cold-Hypoxia-Restraint Multiple Stress Animal Model for Adaptogenic Studies

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## Abstract

Cold-Hypoxia-Restraint (CHR) animal model was developed in our laboratory to study the effect of multiple stresses on normal physiology of rats. This is an established model to study adaptogenic potential of various pharmaceutical interventions. In this model the resistance in fall of core body temperature (CBT) after oral supplementation of an intervention is used as a measure of endurance. CBT of male Sprague-Dawley rats was continuously monitored under multiple stresses using radiotelemetry sensor, surgically implanted in abdomen. The technique was further validated using known adaptogen. Post surgery decline in the weight and consumption of food and water was observed which recuperated within 3 days. Activity counts of rats decreased by upto 66% under CHR stress. Under CHR, 13% more resistance was seen in telemetered rats as compared to those in which rectal probe is inserted. Two known adaptogens *Hippophae salicifolia* and *Hippophae rhamnoides turkestanica* when studied using radiotelemetry gave respectively 80% and 69% resistance to CHR induced hypothermia. This study presents radiotelemetry as an excellent technique to function under hypobaric hypoxia, the condition in which many systems do not function optimally. To the best of our knowledge telemetry is being used for the first time for adaptogenic studies. Consequently adaptogenic studies validate the use of radiotelemetry under CHR. CHR animal model plays a pivotal role in pre-clinical trials and finds its ardent role in multiple stress studies. Radiotelemetry can be employed for evaluating the anti-stress and adaptogenic activities. It ethically reduces the number of experimental animals that can be used for long term studies and give authenticated data. Further experiments are ongoing to study the effect of physiological parameters such as BP, ECG, EMG under the CHR conditions which will give an insight in the multifaceted stress signaling response.

Key-Words: Adaptogen, Cold, *Hippophae*, Hypoxia, Radiotelemetry, Restraint

## Introduction

Stressors such as cold, hypobaric hypoxia and restraint at high altitude lead to decline in physical and mental performance of human beings. Our investigation started with an initiative to study the effect of such stressors on normal physiology of defence personnel deployed at high altitudes. For this purpose in 1990s our laboratory developed a passive experimental model for rats (Ramachandran et al., 1990). The model became famous as Cold-Hypoxia-Restraint multiple stress animal model (CHR). In this model the high altitude conditions were simulated in rats using vacuum generated system leading to decrease in partial pressure of air equivalent to 428 mm Hg in addition to cold (5°C) and restraint stress. Under such conditions the core body temperature (CBT) of the rats could only be measured using rectal probe thermometry.

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CBT is a basic physiological parameter, which is often measured in awaken rodents by rectal probe thermometry (Bae et al., 2007). It is a direct indicator of body's homeostasis in homeotherms. Therefore the need arose of a system that could measure internal temperature in rats without disturbing their physiology (Molčan et al., 2009). It was also realized that measurements of physiological variables in experiments were frequently affected by irregularities resulting from handling of animals. We were in search for a system that could circumvent the use of rectal probes and could also work under hypobaric hypoxia as not many systems were found to be operational under reduced pressure created by vacuum generated systems of CHR. We have been able to achieve this through radiotelemetry system. Radiotelemetry came as a saviour in existing CHR animal model as it is now possible to monitor CBT with minimum handling of experimental animals before and during measurements.

With the use of radiotelemetry known sources of artifacts are avoided and accurate and reliable measurements are assured (Molčan et al., 2009).

A gradual fall in the CBTs of the rats is observed when they are exposed in multiple stressors of CHR. The resistance in the fall of rectal temperature of a homoeothermic animal like rat under CHR after oral supplementation of the herbal agent is used as a measure of endurance. The preference of herbal agents is based upon the hypothesis that plants growing under adverse environmental conditions acquire bio-molecules which help them to sustain in such type of harsh climate. For this reason a number of plants growing at high altitude areas have been screened for their hypoxia and cold resistance inducing activity. Plants belonging to different genera like *Panax*, *Gingko*, *Rhodiola*, *Valeriana* and *Hippophae* and formulations like Composite Indian Herbal Preparations (CHIP-I-III) have been screened for their adaptogenic activities using CHR (Kumar et al., 1999, 2002; Geetha et al., 2003; Saggu et al., 2007; Tulsawani et al., 2010 and Sharma et al., 2012). Thus we have already established the role of CHR to study the endurance promoting activity of herbal agent as an adaptogen.

This study presents the improvisation of CHR using radiotelemetry as a technique, its advantages and validation using adaptation studies. To the best of our knowledge this study reports for the first time the measurement of CBT of a freely moving animal under cold and hypobaric hypoxia. This is also for the first time that such system is being used for determination of adaptogenic potential of aqueous leaf extracts of two Seabuckthorn species, *Hippophae salicifolia* (SBT-1) and *Hippophae rhamnoides turkestanica* (SBT-5), plants. In our previous study (Sharma et al., 2015) the remarkable adaptogenic potential of these two plants have already been established. Consequently they have been selected to endorse the utility of radiotelemetry for adaptogenic studies.

## Material and Methods

### Experimental Animals

The study was approved by the Animal Ethical Committee of our institute in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) of the Government of India.

Sprague–Dawley inbred male rats from the experimental animal facility of the Institute were used for the study. The rats were maintained inside polypropylene cages under a controlled environment in the institute's animal house at 22 ± 1°C, 55 ± 1% humidity and 12 h light–dark cycle. The rats had free access to standard animal food pellets and water.

Daily record of water and food intake of the animals issued from animal facility is maintained. The surgery room is fumigated using 40% formaldehyde solution. Then it is sealed for 48 hours. Further it is neutralized using 10% ammonia solution 3 hours prior surgery.

### Experimental Model

The study was conducted in two parts using CHR animal model (Fig.1A):

(I) Rectal probes (RP) study- Rats were divided into 3 groups of 4 rats each:

(1) Rats administered with distilled water, (2) and (3) Rats administered with SBT-1 and 5 respectively at an optimized dose of 100 mg/kg body weight. All the oral treatments were done 30 minutes prior to CHR exposure.

For this study the rats in restrained state were exposed to cold (5°C) and hypoxia (428 mmHg), equivalent to an altitude of 4572 m. The rectal probe (Fig.1B) was inserted 2 cm pass the rectum and retained there with the help of adhesive plaster. The rectal temperature ($T_{rec}$) of the rats was monitored throughout the experiment after an interval of one minute, by using a 16-channel Iso-thermex Temperature Recorder (Columbus Instrument, Columbus, USA) (Fig.1C). When the rats attained a rectal temperature ($T_{rec}$) of 22±1°C, they were taken out of the decompression chamber.

(II) Implantable telemetry sensors (ITS) study- Radiotelemetry system from the Data Sciences International (St. Paul, Minnesota, USA) was used. The details of DSI system are given in Table 1A. ITS transmit signals to receiver. For this study TA10TA-P40 (Fig.1D) sensor was implanted inside the abdominal cavity of the rats to measure core body temperature (Fig 2A and 2B). The weight of the sensor was 7.25 g and the volume was 3.5 cc. This sensor was suitable for the rats more than 175 g. An additional system of Ambient Pressure Reference (APR) was used for effective working of ITS under hypobaric hypoxia.

8 mature healthy male rats, 15–16 weeks old, weighing 180-250g were used for the ITS study. The details are also given in Table 1B. Animals were anesthetized with Aneket (Ketamine 5%) and Xyloxine (Xylazine 2 %) in the ratio 3:1. For better recovery of the animal, antibiotic and analgesic treatments were given to the animal once in a day for three days. Each animal was kept in the strictly isolated condition, ambient temperature maintained at 25 ± 1˚C, light: dark cycle 12:12 during the entire experiment. Special glucose based diet was provided to the animal during recovery period. Each day post surgery, rats were weighed and

examined for their food and water consumption. When pre-surgical body weight was reached /exceeded and activity established, radiotelemetry measurements were initiated.

**Exposures of ITS study**

After the post-surgery recovery of the animal each animal was subjected to four types of exposures with a minimum gap of 7 days between each exposure. The exposures were coded as (i) Cold-Hypoxia (CH) i.e. without restraint or freely moving animal (ii) Cold-Hypoxia-Restraint (CHR). Both (i) and (ii) were control exposures done after 30 minutes of administration of 0.5 ml water, (iii) administration of aqueous extract of *Hippophae salicifolia* (SBT-1) at the dose of 100 mg/Kg body weight and (iv) administration of *Hippophae rhamnoides turkestanica* (SBT-5) at the dose of 100 mg/Kg body weight 30 minutes prior to CHR exposure coded as CHR+SBT-1 and CHR+SBT-5 respectively.

***Hippophae sp.* Extract Preparation**

Plants SBT-1 and SBT-5 are grown at Seabuckthorn Research Farm of CSK Himachal Pradesh Agricultural University at Kukumseri in Lahaul valley of District Lahaul-Spiti, a semi-arid region of Himachal Pradesh, India at an altitude of 2730 m in sandy loam soil with annual rainfall of 500mm. The minimum temperature during winter is -15°C and maximum temperature during summer is 32°C. The annual recorded snowfall is 150-250 cm. The age of the plant at the time of leaf-collection was 8 years.

The details of extract preparation are given in Sharma et al., 2015. In brief aqueous extracts were prepared using Accelerated Solvent Extractor (ASE). Both the extracts had been analyzed for their *in vitro* antioxidant potential.

**Statistics**

The results were analyzed by one-way ANOVA (analysis of variance) with Sidak's Multiple Comparisons Test using Graph Pad Prism v.6. Differences were considered to be significant when the p values were $< 0.05$.

## Results and Discussion

Daily record of each animal for their weight (Fig. 3A), water and food consumption (Fig. 3B) was kept for the entire period. The weight of the animal reduced abruptly for the first five days after surgery followed by gradual increase and then maintaining at certain level for almost 3 days. Once the animal had attained its pre-surgical weight it was ready for exposure. Each animal was exposed to CH, CHR, CHR+SBT-1 or CHR+SBT-5 but not necessarily in the same order. A gap of almost 7-10 days was kept in between the different exposures. After each exposure a loss of almost 10g in weight was seen in the animal which again regained after 2-3 days. Similar trends were observed for food and water consumption. The food and water consumption were low for few days post-surgery, then maintained at a certain level. However the abrupt decrease in both the values was observed after exposures to CH or CHR. The average consumption of food and water were 20 g/day and 24.75ml/day respectively for the entire period.

The changes in core body temperature under CHR, as measured using rectal probes (CHR-Probes) and telemetry sensor (CHR-Telemetry) are shown in Fig. 4A. Using telemetry it was possible to remove restraint stress therefore the core body temperature measurements was done under exclusive CH conditions (CH-Telemetry) as shown in Fig.4A. When the restraint stress was removed or in a freely moving state it was observed that the rats were able to maintain their core body temperature around 34°C even after two hours of continuous exposure in cold and hypoxia (CH-Telemetry) (Fig.4A). The activity of the rats was also recorded under stressful conditions and it was found out that animal remained active during the first 20 minutes (10-15 counts) and after that the activity declined. However a splurge in activity could be seen after every 15-20 minutes (Fig.4B). It was seen that animal started shivering under Cold stress and tried to remain at one place which could also be seen by the decrease in the activity (0-5 counts) counts after 20 minutes of the exposure.

The CHR-Probe rats took 69 minutes to attain rectal temperature ($T_{rec}$) to 22±1°C from 37°C whereas CHR-Telemetry rats took 78 minutes. Table-2A shows Mean Recording of every 10 Minutes of the core temperature of the rats under CH and CHR stresses. Correlation coefficients between two temperature measurement techniques under different types of exposures are given in Table-2B. There was a significant correlation between the telemetry sensor and rectal probe temperatures, values being 0.999 for all data points taken at 10 minutes interval.

SBT-1 and SBT-5 have already shown significant adaptogenic potential under CHR using rectal probes (Sharma et al., 2015). The results were again reproduced for this study, compared with those obtained using radiotelemetry system and are summarized in Table-3. Using telemetry system also the oral administration of SBT-1 has shown good adaptogenic potential as depicted in Fig. 4C, the delay in the time taken to attain $T_{rec}$ 22±1°C is 140 minutes. The delay was of 131 minutes for SBT-5 (Fig.4D). Thus SBT- 1 gave about 80% resistance to C-H-R induced hypothermia whereas SBT-5 provided about

69 %resistances as compared to control (CHR-Telemetry).

The present study describes the use of implantable telemetry system in a multiple stress animal model. Not many system functions under hypobaric hypoxia as such simulated chambers are made with toughened glass to created requisite vacuum for the animal. In such scenario a wireless system becomes an imperative requirement. We have overcome this challenge by employing radiotelemetry system in CHR. Development of CHR in the 1990s was in itself a great achievement and lot of research data have been generated using this animal model. It was a system that could test the in *vivo* potential of herbal extracts and purified compounds under laboratory conditions. The model was developed in this laboratory for determination of adaptogenic potential of herbal agents. We have improvised this model using radiotelemetry which now can evaluate physiological parameters also under this multiple stress conditions.

High altitude plants like seabuckthorn have always gained special attention due to their ability to grow in the harshest of the environment. To test this was a challenge which we overcame by developing CHR animal model. Many herbal adaptogens have been developed using CHR animal model. Use of radiotelemetry system in CHR had some added advantages over conventional use of rectal probes. The size and shape of implantable transmitter (hermetically sealed) was optimum in order to avoid any compromise of the normal physiological function of the animal (Aydin et al., 2011). Using this system it was possible to remove the restraint stress and study the exclusive effect of cold (C) and hypobaric hypoxia (H) on animals. It was found out that physical restraint of rats at 5°C lead to hypothermia, apparently as a result of an inability of the restrained rat to shiver adequately to maintain a sufficient level of heat production (Butz and Davisson, 2001). One additional factor- hypobaric hypoxia has also contributed towards the hypothermia of the animal by disturbing metabolism. Telemetry signifies the use of data collection from a freely moving and conscious animal in their own familiar environment (Kurtz et al., 2005), thus minimizing the stress and consequent experimental artifacts (Cesarovic et al., 2011). Restraint comes out to be a major stress in our study as by removing this stress animal was able to maintain its core body temperature for a prolonged period of time. Activity recording of the animal can be done under cold and hypobaric hypoxia conditions.

This system has also widened the scope of actual experimental protocols as precise measurements can be obtained during surgery, post surgery and under multiple stresses. Previous reports also supported the use of telemetry system for core body temperature measurements (McKenzie and Osgood, 2004). The significant correlation between the telemetry sensor and rectal probe temperatures shows how closely the values are related at all data points at 10 minutes interval.

By using this system we could also reduce the number of experimental animals by almost 33%. Same animal could be used for different experiments as is done in cross over studies. That diminishes chance of inter animal variability. The data obtained is reproducible, precise and free from external artifacts hence less number of experimental animals used is also justified for statistical analysis. Earlier studies also reported such reduction in animal use (van Acker et al., 2001 ; Pugsley et al., 2008 and Kramer and Kinter, 2003).

Water and food consumptions of the animals were assessed throughout the period. After 4-5 days of surgery the animal started gaining weight and remained active. Initial decrease in the weight might be due to the post-surgery stress to the animal. The decrease consumption of food and water after CHR exposures may be contributed towards the fact that hypoxia also leads to disturbance in the metabolism of animals. Previous studies have demonstrated the stress induced reduction in 24 hour food intake (Rybkin et al., 1997).

This was completely a novel idea of employing radiotelemetry to study efficacy of a herbal plant extract on rat model under multiple stress. Thus to validate this new technique we have selected two extracts whose adaptogenicity we already have proved in our previous study (Sharma et al., 2015). This study has also shown seabuckthorn as good adaptogen. The contributory factor remains to be vital antioxidant present in the plants besides other morphological adaptations.

## Concluion

Radiotelemetry came out to be an excellent system for adaptogenic studies in CHR. This system can be easily employed for evaluating the anti-stress and adaptogenic activity as the results were comparable to those obtained using conventional rectal probes. Using radiotelemetry it was possible to remove the restraint stress and study the activity of the animals under cold and hypobaric hypoxia. Radiotelemetry is a more humane method for investigations involving small animals like rats. It ethically reduces the number of experimental animals that can be used for long term studies and give authenticated data.

Further experiments are ongoing to study the effect of physiological parameters such as BP, ECG, EMG under the CHR conditions which will give an insight in the multifaceted stress signaling response.

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**Table 1A: Telemetry system for Radiotelemetry Recordings**

| **Telemetry System from DSI** |
|---|
| **TA10TA-P40- sensor- 7.25g, 3.5cc** |
| **Radio AM 535-1605 KHZ (w/batteries)** |
| **Magnet (370-0034)** |
| **RPC-1 Receiver** |
| **Data Exchange Matrix** |
| **Ambient Pressure Reference (Model APR-1) for Hypoxia Chamber** |

**Table 1B: Surgery preparation and Medication for abdominal radio transmitter implantation in rats**

| **I. Surgical Preparation Equipments** | **II. Medication used pre -surgery** | **III. Medication used post-surgery** |
|---|---|---|
| **1. Electrical hair clipper, MOSER, Germany**<br>**2. Suture kit-Johnson-Johnson-Non colored needle-thread combination**<br>**3. 0.9% Saline S0dim chloride-Merck, Millipore water-Autoclaved**<br>**4. 1ml syringe**<br>**5.5ml syringe**<br>**6. Sterile Surgical blade No.24**<br>**7. Needle, Hypo, 14-Gauge,1.5’’** | Anaesthesia antagonist<br>KETAMINE-<br>Ketamine Injection IP-<br>50 mg/ml at 80mg/Kg body wt. | Antibiotic<br>RANBIOTIC-<br>Gentamicin sulphate Injection IP. 40mg/ml |
| **Alcohol disinfectant**<br>**Absolute Ethanol, Merck** | Anaesthesia agonist-<br>XYLAXIN-<br>Xylazine Injection IP-<br>20 mg/ml at 10mg/Kg body wt. | Analgesic<br>VOVERAN –<br>Diclofenac Injection IP. 75mg/ml |
| **Povidone-Iodine solution IP. 5% w/v-BETADINE Microbicidal solution** | | |

**Table 2A: Mean Recordings of core temperature every 10 minutes under CH and CHR stresses using rectal probe and radiotelemetry implant**

| Mean Recording of core temperature | CH-TELEMETRY | CHR-PROBE | CHR-TELEMETRY |
|---|---|---|---|
| 0 | 37.41 | 36.58 | 37.27 |
| 10 | 37.12 | 34.54 | 35.83 |
| 20 | 36.15 | 32.14 | 33.33 |
| 30 | 35.50 | 29.62 | 31.08 |
| 40 | 35.02 | 27.45 | 29.02 |
| 50 | 34.63 | 25.88 | 27.12 |
| 60 | 34.54 | 23.96 | 25.47 |
| 70 | 34.41 | 22.52 | 24.32 |

**Table 2B: Correlation coefficient between two temperature measurement techniques under different types of exposures**

| Exposures Types | CH/CHR-T | CH/CHR-P | CHR-T/CHR-P |
|---|---|---|---|
| **Correlation coefficient** | 0.982 | 0.981 | 0.999 |

**Table 3#*: Adaptogenic activity of seabuckthorn leaf-extracts after single dose (p.o.) of 100 mg/Kg. body weight using both Rectal probes and Telemetry**

| Experiment Code | $T_{rec}$23°C | % Change |
|---|---|---|
| **CHR-Probe** | 69±1.88 | - |
| **CHR-Telemetry** | 78±2.54 | - |
| **CHR+SBT-1-Probe** | 137±0.41* | 99 |
| **CHR-SBT-1-Telemetry** | 140±17.64# | 80 |
| **CHR+SBT-5-Probe** | 125±4.97* | 81 |
| **CHR+SBT-5-Telemetry** | 131±8.09 | 69 |

# Values are mean ± SE of 6 rats in Probe group and 8 rats in Telemetry group and expressed as percentage change.
* $P<0.05$, compared with CHR-P # $P<0.05$, compared with CHR-T.

Figure 1
A. Cold-Hypoxia-Restraint (CHR) Animal model
B. Rectal probe.
C. Isothermex & Restrainers
D. TA10TA-F40 implant.


Figure 1.

Figure 2
A. Radiograph of rat showing location of implanted telemetry transmitter- TA10TAF40 (Ventral view).
B. Radiograph of rat showing location of implanted telemetry transmitter- TA10TAF40 (Lateral view)

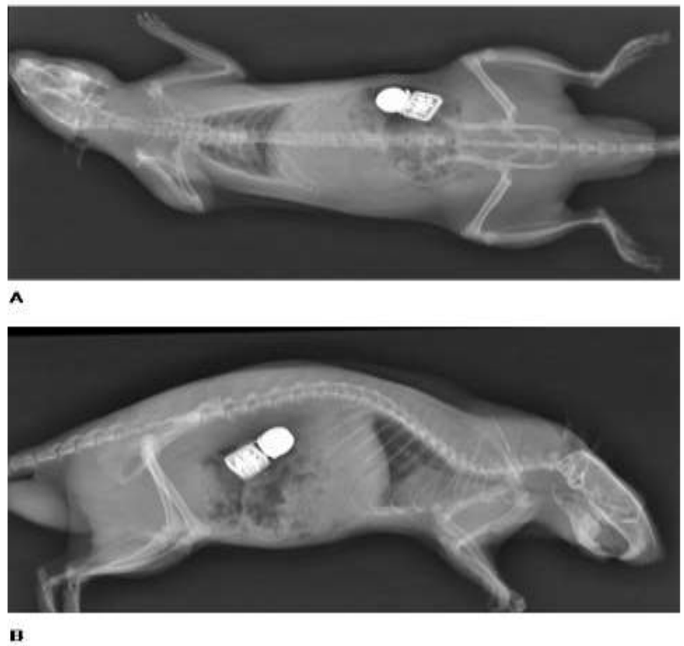

Figure 2.

Figure 3
A. Weight change of the animal post-surgery during the entire period.
B. Consumption of water and food by the animal post-surgery for the entire period.

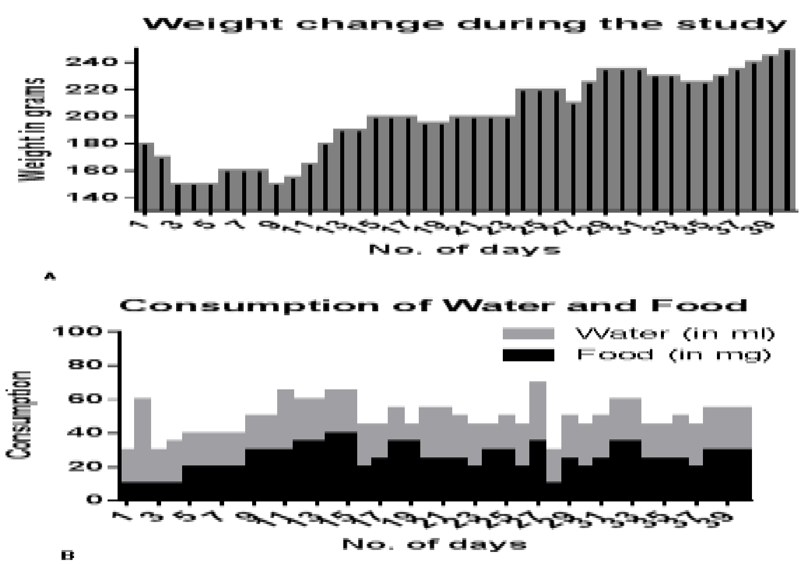


Figure 3

Figure 4
A. Mean effects of Cold-Hypoxia (CH) and Cold-Hypoxia-Restraint (CHR) stress on core and rectal temperature of rats.
B. Mean effects of Cold-Hypoxia stress on the activity of the freely moving animal.
C. Mean effects of Cold-Hypoxia-Restraint stress on core temperature of rats (CHR) and the effect followed by SBT-1 administration (CHR+SBT-1) using radiotelemetry.
D. Mean effects of Cold-Hypoxia-Restraint stress on core temperature of rats (CHR) and the effect followed by SBT-5 administration (CHR+SBT-5) using radiotelemetry.

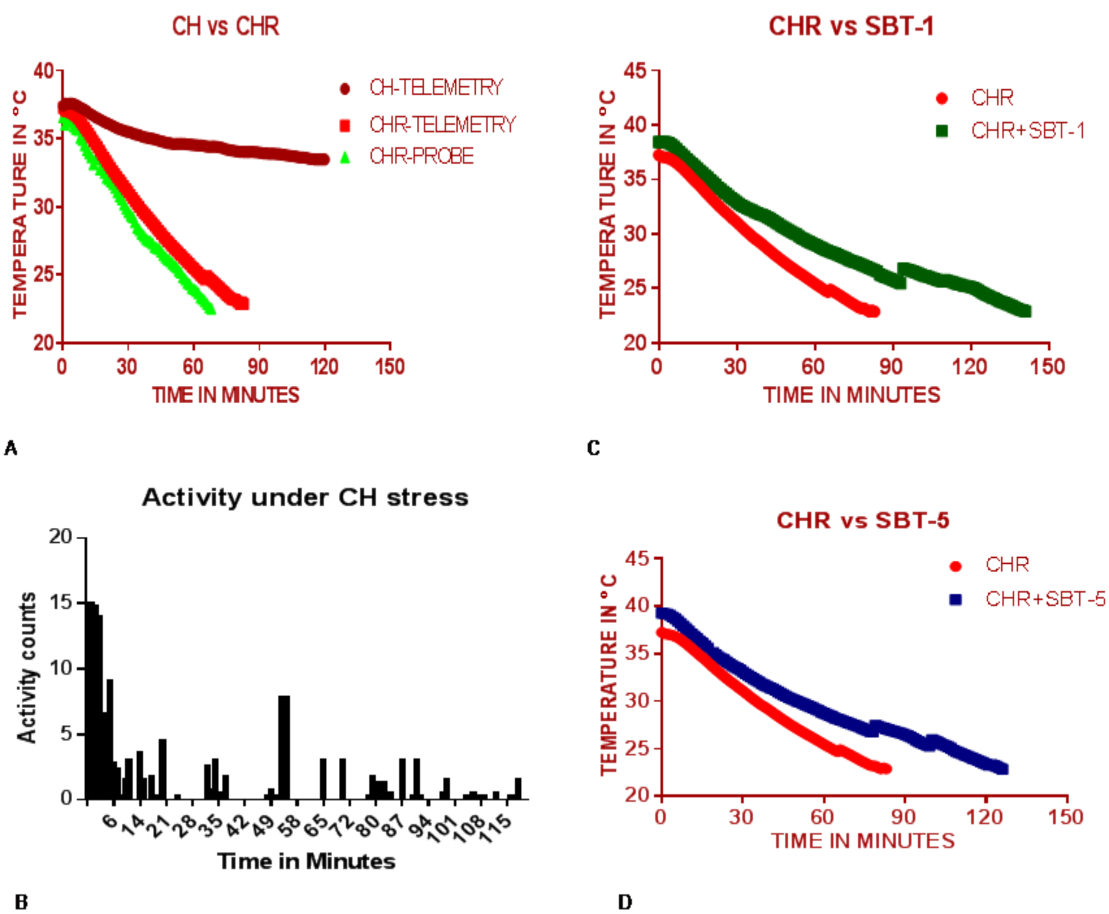


Figure 4

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