RESEARCH PAPER

Evaluation of Phototherapy Devices Used for Neonatal Hyperbilirubinemia

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Objective: To compare phototherapy devices based on their physical and photo-biological characteristics *viz* spectral properties, maximum and mean irradiance, treatable percentage of body surface area, decay of irradiance over time and *in vitro* photoisomerisation of bilirubin.

Design: *In vitro* experimental study.

Setting: Ocular pharmacy laboratory at a tertiary care hospital.

Methodology: All the characteristics were measured at a fixed distance of 35 cm from one compact fluorescent lamp (CFL) and three light emitting diode (LED) phototherapy devices in a dark room with an irradiance of <0.1μW/cm^2/nm. Estimation of products of *in vitro* photoisomerisation was done using liquid chromatography - tandem mass spectroscopy (LC-MS/MS).

Results: The emission spectral data were comparable between the phototherapy devices. The devices, however, differed in their maximum irradiance with the spot and indigenous LED units having the highest and lowest values, respectively (56.5 and 16.8μW/cm^2/nm). The mean irradiance – measured in 5x5cm grids falling within the silhouette of a term baby – of the spot and improvised LED devices were low (26.8μW/cm^2/nm and 11.5μW/cm^2/nm, respectively) possibly due to unevenness in the irradiance of light falling within the silhouette. There was a significant difference in the amount of bilirubin left after exposure to light over a 2-hour time period (% reduction of bilirubin) among the four devices (*P*=0.001); at 120 minutes after exposure, the amount of bilirubin left was lowest for the CFL (16%) and spot LED (17%) devices and highest for the indigenous LED unit (41%).

Conclusions: The four phototherapy devices differed markedly in their physical and photobiological characteristics. Since the efficacy of a device is dependent not only on the maximum irradiance but also on the mean irradiance, rate of decay of irradiance, and treatable surface area of the foot print of light, each phototherapy device should have these parameters verified and confirmed before being launched for widespread use.

Key words: *Efficacy, Neonate, Jaundice, Phototherapy, Compact fluorescent tube, Light emitting diode.*

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Phototherapy should be regarded as a drug, with an appropriate dose and duration, used to manage hyperbilirubinemia in neonates. There is no standardized method for reporting phototherapy dosages in the clinical practice. The 'dose' of phototherapy would depend upon the device characteristics such as emission spectral data, maximum irradiance, mean irradiance, treatable percentage of body surface area (BSA), age of the light source, and possibly the amount of formation of photoisomers from bilirubin.

Accompanying Editorial: Page 681-82.

Currently, no guidelines are available for measuring the efficacy of different phototherapy devices used in the country. Bench studies from the West have shown widely varying efficacy of these devices [1-4]. One of the few studies from India that evaluated the phototherapy devices used in different hospitals of a major city found only 31% of the units to be providing an acceptable level of irradiance (at least 15μW/cm^2/nm) and a meager 8% of the devices to have the recommended special blue lights [5].

Neonates with hyperbilirubinemia treated with suboptimal devices may require prolonged phototherapy or even exchange transfusion because of failure of phototherapy. There is a need to standardize the phototherapy devices so that effective devices are used for the management of neonatal hyperbilirubinemia. The present study was designed to evaluate and compare different phototherapy devices and also to develop standardized methods for evaluation.

METHODS

We tested four different phototherapy devices: (*i*) Spot LED (Phoenix Medical Systems Pvt Ltd, India); (*ii*) Indigenous light emitting diode (LED) (Photolux, SriChakra Scientifics, India); (*iii*) Improvised LED (Bilitron Fanem Inc, Brazil); and (*iv*) Compact fluorescent lamp (CFL) unit (Phoenix Medical Systems Pvt Ltd, India). The experiments were conducted in a dark room with irradiance of <0.1 μW/cm^2/nm and at fixed distance of 35 cm. The devices tested, except for indigenous LED, were brand new devices. The spot LED device has a high intensity (40 W) LED bulb encased in a cup shaped enclosure fixed on a pedestal. The indigenous LED device consists of green LED (33 bulbs) arranged in three center rows and multiple rows of blue LED (176 bulbs) flanking the green on either side. The improvised LED unit consists of 5 high intensity LED bulbs mounted on a mobile pedestal. A fan within the unit helps to dissipate the heat. The CFL phototherapy device consists of six 18 W double folded (8 inches) special blue CFL encased in a rectangular box fitted with a light reflector.

Measurement of surface area: A white spacer board made up of packing material was cut to size 60×30 cm and a white paper having vertical and horizontal lines forming grid size of 5×5 cm was pasted on it. Silhouette of a term (gestational age 38 wks) appropriate for gestational age baby was then traced on the white paper. The surface area of the silhouette was 780 cm^2. The size of the board was similar to that recommended by International Electrotechnical Commission (IEC) which defines the "effective surface area" as the intended treatment surface that is illuminated by the phototherapy light [6].

Comparison of peak emission spectra: We measured the peak emission spectra of the lamps of the four devices at Nanophotonics Division, IIT Delhi, using a portable HR2000CG-UV-NIR optical spectrum analyzer (Ocean Optics Florida, USA). This high resolution spectrometer has a detector that covers the 200-1100 nm wavelength range and interfaces to a personal computer via USB 2.0 port. The phototherapy devices were transported to the laboratory and spectral data were recorded.

Comparison of spectral irradiance: Spectral irradiance was measured using spectroradiometer (Biliblanket Meter II; Ohmeda Medical, GE Health care, USA). This instrument is a fixed spectroradiometer capable of picking up irradiance between 400-520 nm with peak sensitivity at 450 nm. The measurements were done at the center of the measuring surface and at four perpendicular peripheral points (at a distance of 15 cm [breadthwise] and 30 cm [lengthwise] from the center) once a day for three consecutive days and the average of the three readings was taken.

Comparison of mean irradiance: The spacer board was placed under the different phototherapy systems and the spectral irradiance was measured in each of the 5x5cm grid falling within the silhouette of the baby. The mean irradiance was determined by averaging the spectral irradiance obtained in each of these grids.

Decay of spectral irradiance: All the phototherapy devices were switched on and allowed to run continuously for a total duration of one month. The instruments were connected through a voltage regulated power source to avoid fluctuations in power supply. Spectral irradiance was checked daily for a period of one month at the center of the field

and at the four peripheral perpendicular points. The decay of spectral irradiance over time was calculated.

Treatable body surface area: As it is difficult to calculate the body surface area of the irregular shaped term baby outline, we used an indirect method suggested by Vreman, *et al.* [1].

In vitro quantification of bilirubin and confirmation of photoisomers: Thermo Finnigan high performance liquid chromatographic (LC) system (Thermo Electron Corp, Waltham, MA, USA) with PDA detector controlled by ChromQuest (Ver.4.5) software was used to elute the analyte. Electron spray ionization technique in positive mode was applied using Turbo Ionspray source (Applied Biosystems, Foster city, CA, USA) in a 4000 Q trap tandem mass spectroscopy (MS/MS) (MDS SCIEX, Applied Biosystems,Foster city, CA, USA). Tandem mass spectroscopy was controlled using Analyst (Ver.1.4.2) software.

HPLC conditions: For the analytical separation, hydrophilic interaction chromatography technique was employed using ZIC-HILIC column (50x4.6mm, 3.5µm particle size; Merck SeQuant AB, Umea, Sweden).

The samples amounting to 20µL were added to 200µL of extraction solvent (70% acetonitrile containing 0.1% formic acid) containing 250ng of homatropine (internal standard) and subjected to vortex for 1 min and loaded into the HPLC autosampler for analysis. Samples were injected at the volume of 20µL and each run was optimized for 3 minutes. Serially diluted standards varying from 0.98ng/mL to 250ng/mL were injected in triplicate and used for quantification. Interday and Intraday variations for the above standards were found to be within the limits of CV<10%. The LC-MS/MS data was analyzed using 'Analyst' (Ver. 1.4.2).

Confirming the production of photodegraded products having similar molecular weights as that of bilirubin: The photoisomerization of bilirubin was analyzed by using standard bilirubin diluted from the stock solution reaching the concentration of 100ng/mL. Three 1.2 mL vials (using auto sampler vials of HPLC) containing 1mL of the methanolic bilirubin solution (concentration of 1 µg/mL) were placed horizontally on a white background kept at a distance of 35 cm from the spot LED lamp at the point of maximum irradiance (previously determined by using fixed spectroradiometer). 10 µL of above solution was aspirated prior to and after 2, 12, 22, 37, and 67 minutes of exposure to light and subjected for analysis using LC/MS/MS using the conditions stated above. The chromatogram thus obtained is depicted in ***Fig.*1**.

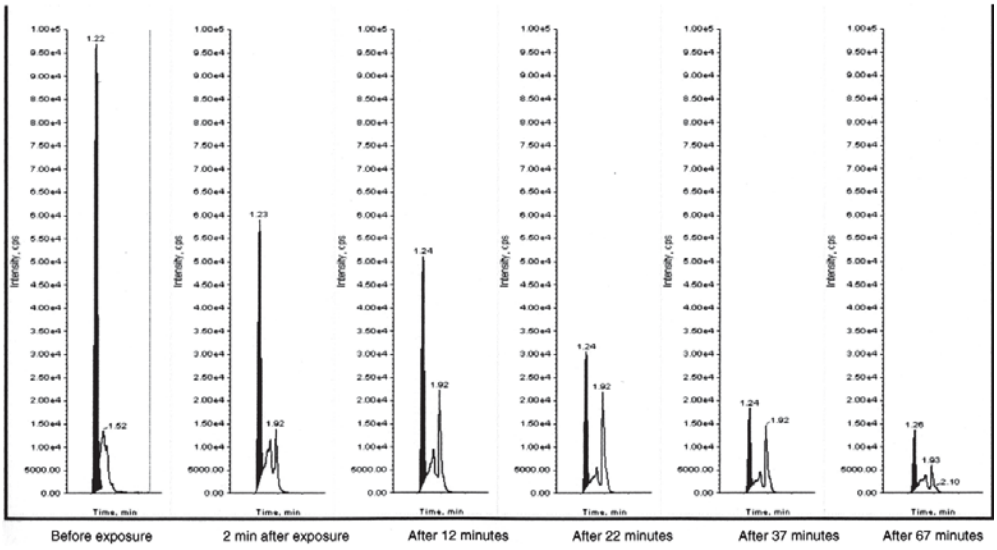

FIG.1 *LC-MC/MS graph showing the bilirubin and photoisomer peaks before and at different time points after exposure to spot LED phototherapy light.*

TABLE I COMPARISON OF DIFFERENT PHOTOTHERAPY DEVICES

Phototherapy device	Emission spectral data (nm)			Maximum irradiance ($\mu W/cm^2/nm$) (Mean ± SD)	Mean irradiance# ($\mu W/cm^2/nm$) (Mean ± SD)	Decay of irradiance ($\mu W/cm^2/nm/day$)	Area of foot print of light (cm^2)
	Peak emission spectra	Band width*	Spectral range				
Spot LED	463	35	420-520	56.5 ± 1.9	26.8 ± 1.3	0.34	755
Indigenous LED	464,*517	70	430-586	16.8 ± 0.1	10.8 ± 0.3	0.14	1800
Improvised LED	456	28	420-520	39.7 ± 0.6	11.5 ± 0.3	0.04	2200
CFL	450	60	400-550	37.5 ± 0.3	26 ± 0.1	0.32	1800

** Band width: Absolute difference between the wavelengths at which the spectral radiant intensity is 50 percent of the maximum power; # Mean irradiance: Average of irradiance of the light falling within the silhouette of the neonate.*

TABLE II COMPARISON OF SPECTRAL IRRADIANCE OF DIFFERENT PHOTOTHERAPY DEVICES AT THE CENTER AND AT FOUR PERIPHERAL PERPENDICULAR POINTS ($\mu w/cm^2/nm$) (MEAN ± SD)

	East	West	Center	North	South
Spot LED	16.8 ± 0.9	22.3 ± 1.2	56.5 ± 1.9	0.8 ± 0.1	0.9 ± 0.1
Indigenous LED	9.8 ± 0.1	10.3 ± 0.2	16.8 ± 0.1	3.5 ± 0.1	2.3 ± 0.0
Improvised LED	6.1 ± 0.2	3.8 ± 0.2	39.7 ± 0.6	0.4 ± 0.1	1.1 ± 0.1
CFL	25.9 ± 1.1	27.9 ± 2.5	37.5 ± 0.3	19.5 ± 1.1	16.7 ± 0.9

East, West, North, South – four peripheral perpendicular points at a distance of 15cm breadth-wise (i.e. East/West) and 30cm lengthwise (i.e. North/South) from the center.

Comparative evaluation of phototherapy devices using bilirubin: Similar to the method used for the confirmation of the HPLC separation of bilirubin from photoconverted products, 1.2 mL vials contain-ing 1 mL of methanolic solutions of bilirubin at the concentration of 1µg/mL (serially diluted from the stock solution) were placed under all phototherapy devices at the point of maximum irradiance at a distance of 35 cm and 10 µL of the bilirubin solution was aspirated from all the vials before and after 15, 30, 45, 60, 90 and 120 min of exposure to light. All the light sources were switched on at least 1 hour prior to the experiment. The same bilirubin solution kept in the dark served as a control during the experiment.

Data entry was done using Excel 2007 (Microsoft, Redmond, WA, USA). Analysis was done by using Excel 2007 and SPSS 15.0 version for Windows. Data were presented as mean (SD) or number (%) as appropriate. Friedman non-parametric two-way ANOVA was used to compare the percentage reduction of bilirubin noted over a time period with the four phototherapy devices. A *P* value of <0.05 was considered as statistically significant.

RESULTS

The device characteristics of the four phototherapy devices tested are summarized in ***Table* I**. The spot LED and improvised LED devices had similar range of emission spectra but the peak emission spectra was slightly different (spot LED, 463nm; improvised LED, 456nm). The indigenous LED device showed double peak (464nm and 517nm) in the emission spectra due to the presence of blue and green lamps. The spectral data for special blue CFL bulbs (Philips Electronics India Pvt Ltd, India) showed a peak emission spectrum of 450 nm with a slightly wider spectral range than the spot and improvised LED devices (***Fig*. 2**).

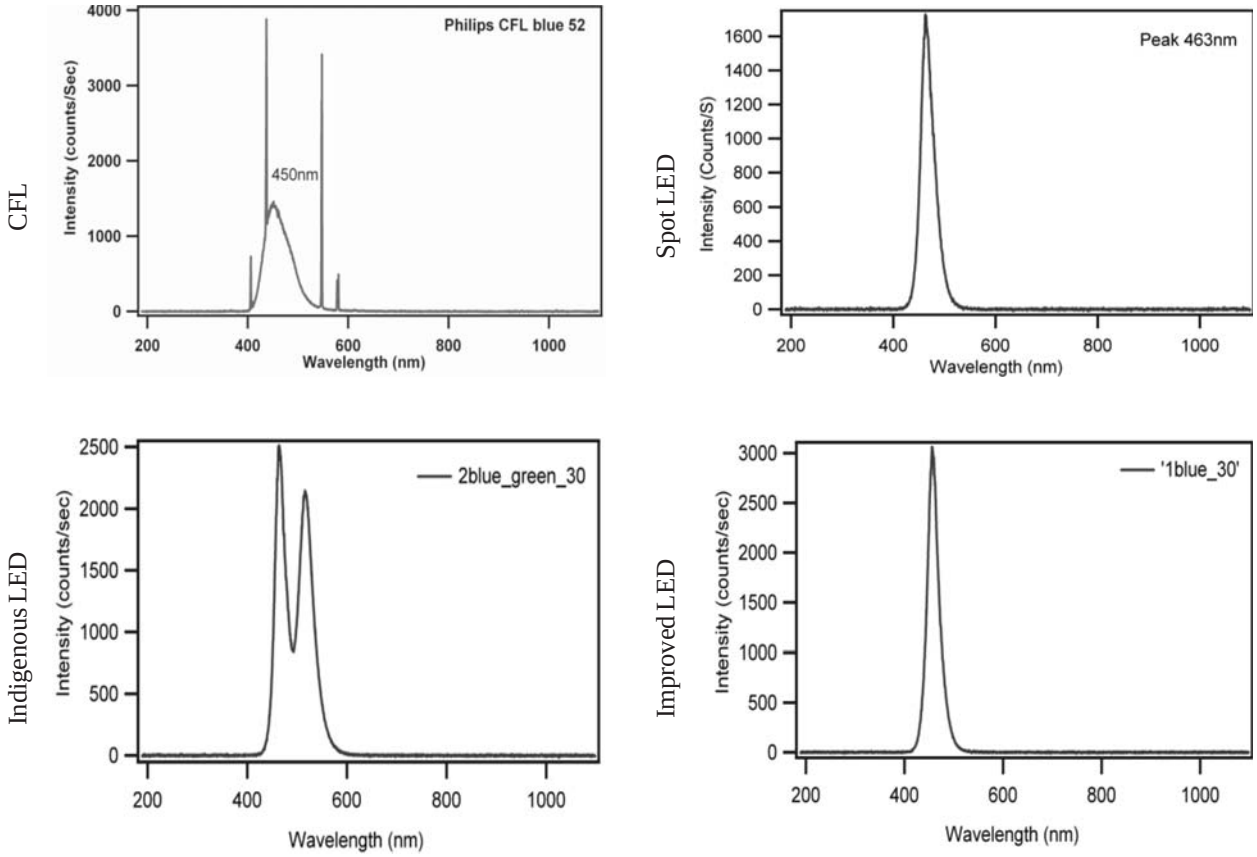

FIG. 2 *Emission spectra of different lamps.*

Maximum irradiance: The average maximum irradiance at the center and at four different perpendicular points at the periphery of the different devices is shown in ***Table* II**. The spot LED and the indigenous LED devices had the highest and lowest maximum irradiances, respectively (56.5 and 16.8 μW/cm^2/nm). Improvised LED and CFL units had almost equal irradiance at the center. In contrast to the high maximum irradiance observed at the center, the mean irradiance (measured in those grids that fall within the silhouette of the term baby) of the spot and improvised LED devices was low (***Table* II**). This unevenness in the distribution of irradiance is depicted graphically in the surface irradiance plots. The CFL device had more uniform distribution of irradiance (***Fig*. 3**). The decay of irradiance over a period of one month was highest in the spot LED system (***Table* II**). Improvised LED system had the least decay of irradiance over time.

The 2D surface area of the term baby silhouette was 780 cm^2. The surface area of the foot print of the spot LED (diameter=31cm), indigenous LED, improvised LED and CFL devices were 755, 1800, 1800, and 2200 cm^2, respectively. While the foot prints of the CFL, indigenous and improvised LED lights covered the 2D silhouette of the term baby completely (treatable surface area of 100%), the foot print of the spot LED light covered only 55% of the term baby silhouette.

There was a significant difference in the amount of bilirubin left after exposure to light over a 2-hour time period (% reduction of bilirubin) among the four devices (P=0.001). At 15 minutes after exposure, only 50% of native bilirubin was left in the sample, the amount was comparable for all the devices except for the indigenous LED unit (***Fig.* 2**). At 120 minutes, the amount of bilirubin left was lowest for the CFL (16%) and spot LED (17%) devices and highest for the indigenous LED unit (41%). The rate of photoconversion reached a plateau after 60 min of light exposure with all the four devices. The percent reduction of bilirubin observed with CFL device after 60min of exposure was higher than that with improvised LED despite a higher maximum irradiance of the latter (***Fig.* 2**).

DISCUSSION

The phototherapy devices differed in their physical and photobiological properties. None of the devices

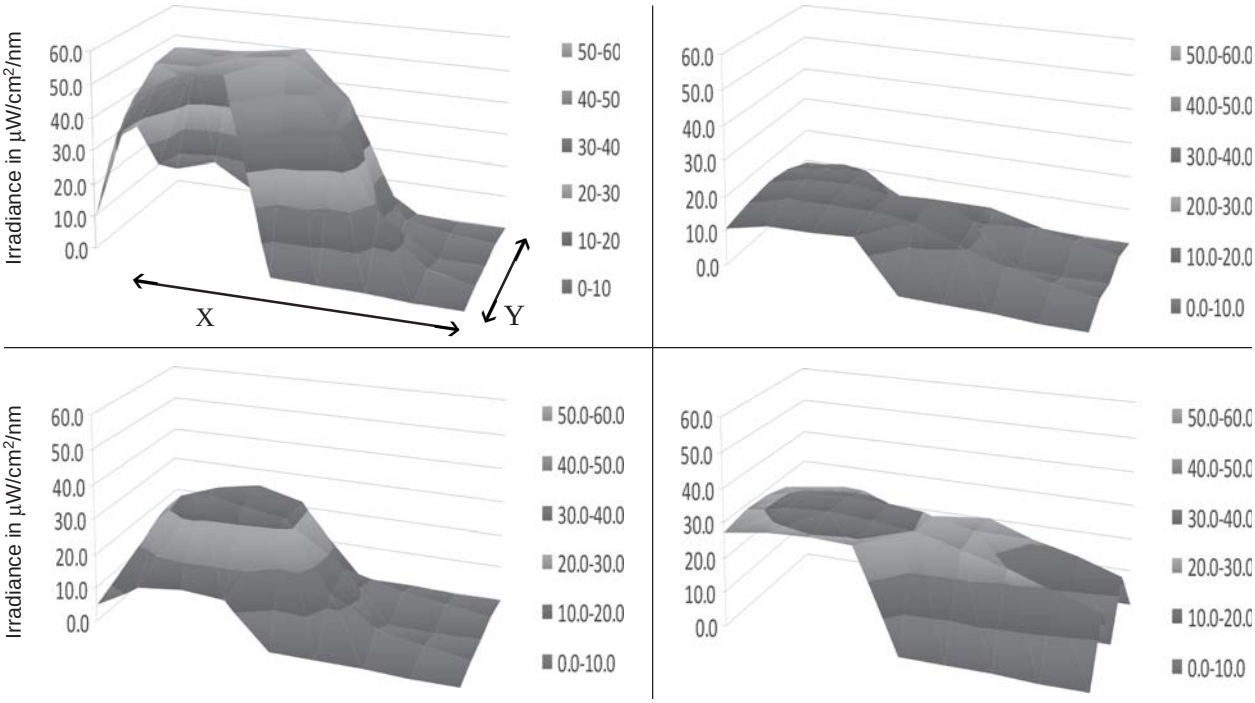

FIG.3 *Surface irradiance plots for different phototheray devices. (a) Spot LED, (b) Indigenous LED, (c) Improvised LED, (d) CFL; X=60 cm, Y=30cm (size of the spacer board used)*

WHAT IS ALREADY KNOWN?

- Phototherapy devices differ in the maximum irradiance.

WHAT THIS STUDY ADDS?

- Phototherapy devices also differ in other key physical and photobiological characteristics that influence the efficacy of the device.

tested showed harmful spectrum in UV or IR range. The emission spectral ranges of all the blue LED bulbs were narrow with peak emission spectrum very near to the peak absorption spectrum of bilirubin. This characteristic has been well emphasized previously by Vreman, *et al*. [7].

The irradiance at the center of the spot LED and the improvised LED devices were high, the significance of which is not clear given that bilirubin photoconversion could stagnate after certain level of irradiance [8]. The existence of such saturation point is, however, still debated. The uneven distribution of irradiance across the area of exposure led to a drop in the mean irradiance to almost 50% and 25% of the peak irradiance in spot and improvised LED, respectively. In addition, the foot print of spot LED covered only 55% of the two-dimensional body surface area. This has the potential to reduce the overall efficacy of the phototherapy device. The concentration of irradiance centering on a restricted area of foot print of light makes it necessary for the healthcare provider to ensure that the baby and device are in proper alignment. The other two devices, CFL and indigenous LED devices, had wider distribution of irradiance across the foot print of light.

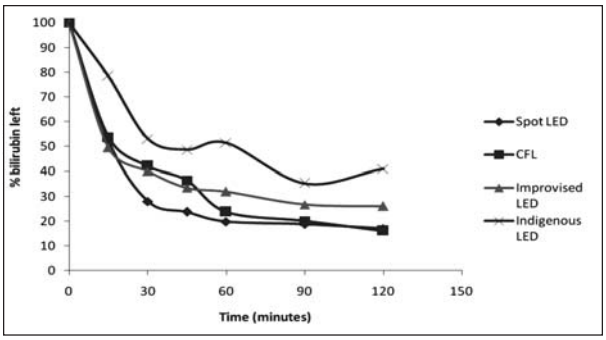

FIG. 4. *Percentage of bilirubin left over (in vitro) after exposure to light with different devices.*

The decay of irradiance and thus the life span of any bulb will depend on the amount of usage and on factors like operating voltage, manufacturing defects, exposure to voltage spikes, frequency of cycling on and off and ambient operating temperature. The phototherapy bulbs showed decline in irradiance over a period of time more so for the spot LED and CFL lamps. We presume higher consumption of amperage and ineffective cooling of bulbs in CFL, and ineffective cooling in spot LED devices when compared to other LED devices could have contributed to this finding.

In vitro formation of lumirubin as a surrogate marker for determining the efficacy of the device was studied previously and neoBLUE LED was demonstrated to be superior [9]. Ennever, *et al*. [10] showed much earlier that the tungsten halogen lamps and special blue lamps generated higher lumirubin levels in comparison to those with broad spectrum like the day light lamps. In our study, though the predominant photoconversion product was estimated (formation of which was linear in the initial part but subsequently had a plateau), we could not specify as to whether the estimated product was lumirubin (structural isomer) or a configurational isomer (both have same molecular weights).

All the devices displayed a linear and similar fall in the bilirubin levels in the initial phase of the study except the indigenous device which demonstrated linear but a slightly delayed fall. In general, the percent reduction of bilirubin was more for the devices with higher maximum irradiance save for the CFL unit which resulted in a higher rate of bilirubin degradation than the improvised LED despite having a slightly low maximum irradiance. This observation is intriguing and is indeed difficult to explain as these data are based on exposure of single sample. The experiment needs to be replicated to generate a robust conclusion and

extrapolation of these data to *in vivo* environment is difficult as it is a more dynamic environment with continuous formation of bilirubin and excretion of photoproducts.

This study is the first of its kind in India. There are no studies from our country which have looked at almost all the parameters that affect the efficacy of the phototherapy devices in an *in vitro* scenario. The method of estimation of bilirubin and its photo-products using LC MS/MS technology is a novel high precision technique. This method used a new technique "hydrophilic interaction chromatography" (HILIC) to resolve hydrophobic bilirubin from its isomers having similar molecular weights.

The limitations of the study were use of fixed band irradiance meter with its attendant limitations but as it estimates irradiance within the therapeutic wavelength range, this should be the appropriate device [11,12]. The method used for mapping irradiance across the foot print of light may not have been perfect but conformed to standards laid by Vreman, *et al.* [1], but still matched what would be relevant for clinical practice.

In conclusion, the available phototherapy devices differed considerably. Combination of character-istics as enlisted in this study should be considered *in toto* before judging the efficacy of the unit. An ideal device should have a maximum and mean irradiance of >30μW/cm^2/nm with the foot print of the light covering an area of at least 60×30cm and distribution of irradiance being uniform across the foot print of light, have least decay of irradiance, and have high rate of bilirubin degradation. CFL had many if not all the characteristics in this *in vitro* study. Knowledge about *in vivo* performance of these phototherapy devices and estimation of photoisomers would further help in characterizing the efficacy of different phototherapy devices. There is a need for regulatory bodies to define standard guidelines to ensure that only efficacious phototherapy devices are marketed.

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Contributors: AKD conceived and designed the study and revised the manuscript for important intellectual content. He will act as guarantor of the study. SS conducted the experiments, analyzed the data and drafted the paper. MJS, RA and VKP provided inputs regarding the design and revised the manuscript for intellectual content. Emission spectral data was recorded by PK and was supervised by GVP. TV and his team designed the methodology to isolate and quantify the bilirubin and photoisomers using LC-MS/MS. The final manuscript was approved by all authors.

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Competing interests: The phototherapy devices were supplied free of cost by Phoenix Medical systems Pvt Ltd,Chennai, India (CFL and LED Spot phototherapy unit); SriChakra Scientifics, Hyderabad. India (Indigenous LED phototherapy "Photolux"); and Fanem Inc, Brazil (Improvised LED Phototherapy). None of the manufacturers had any role in study design, collection of data, analysis, and interpretation of results.

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