OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2016.371.380

Research Article
Light Emitting Diodes (LED) Irradiation Treatment Ameliorates Hyperbilirubinemia in Obstructive Jaundice in Laboratory Animals

[1]Amit D. Kandhare, [1]Anwesha A. Mukherjee, [2]Swanand D. Pasalkar, [3]Sandesh A. Ghate and [1]Subhash L. Bodhankara

[1]Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, 411038 Maharashtra, India
[2]Sanjeevani Enterprises, Pune, 411052 Maharashtra, India
[3]VIT University, Vellore, 632014 Tamilnadu, India

Abstract

Background: Hyperbilirubinemia is a most leading cause of neonatal readmission condition and occurred due to rise in the level of unconjugated bilirubin in the blood. Phototherapy is widely used for the treatment of neonatal hyperbilirubinemia. **Objective:** To study the effect of edixeon S series power Light Emitting Diodes (LED) on obstructive jaundice induced hyperbilirubinemia in laboratory animals. **Material and Methods:** Hyperbilirubinemia was induced in male Wistar rats (180-220 g) via ligation of common bile duct. Rats were exposure to LED (low (0.06 W m^{-2}) and high (1.0 W m^{-2}) irradiation for 5 h for 3 days after recovery from surgery. Control rats were exposed to normal day light for 3 days. After 24 h of 3 days of exposure, blood was withdrawn under anaesthesia and they were sacrificed by cervical dislocation to collect the hepatic tissue. Various biochemical and histopathological parameters were evaluated in serum and hepatic tissue. **Results:** The LED irradiation (low and high) treatment significantly ($p<0.01$ and $p<0.001$) decreased serum albumin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphates (ALP), gamma-glutamyl transferase (GGT), total bilirubin and direct bilirubin levels as compared with control treated rat. Decreased level of hepatic superoxide dismutase (SOD) and glutathione peroxidase (GSH) were significantly ($p<0.01$ and $p<0.001$) increased by LED irradiation (low and high) treatment. It also significantly ($p<0.01$ and $p<0.001$) reduced the elevated level of malondialdehyde (MDA) and Nitric Oxide (NO) levels in hepatic tissue. Histological alternation induced in liver was also reduced by LED irradiation. **Conclusion:** The LED irradiation treatment showed inhibition in the elevated bilirubin levels via modulation of hepatic biomarkers as well as elevated oxidative stress. The use of edixeon S series power LED may be beneficial in terms of low cost of LED emitters and power requirements which can make this LED phototherapy a useful and possibly a preferred modality for the treatment of neonatal jaundice.

Key words: Hyperbilirubinemia, irradiation treatment, jaundice, light emitting diodes, oxidative stress, phototherapy

Received: September 25, 2016 Accepted: November 05, 2016 Published: November 15, 2016

Citation: Amit D. Kandhare, Anwesha A. Mukherjee, Swanand D. Pasalkar, Sandesh A. Ghate and Subhash L. Bodhankara, 2016. Light Emitting Diodes (LED) irradiation treatment ameliorates hyperbilirubinemia in obstructive jaundice in laboratory animals. Pharmacologia, 7: 371-380.

Corresponding Author: Subhash L. Bodhankar, Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune, 411038 Maharashtra, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Today, the most common cause of neonatal readmission to the hospital is hyperbilirubinemia[1-4]. Hyperbilirubinemia is an exceedingly common complication for new-born babies. Almost 50% terms and 80% of preterm are getting high level of bilirubin and resolve spontaneously during the 1st week of life[5]. The normal level of Total Serum Bilirubin (TSB) level in full-term infants usually is 6-8 mg dL^{-1} by 3 days where as in premature infants the peak may be 13 mg dL^{-1} TSB on the 5th day of life. However, without any specific abnormality of bilirubin metabolism possibly rising over 15 mg dL^{-1}. Due to the limited ability of new-born to metabolism these elevated bilirubin thus it's accumulated in the form of unconjugated bilirubin. The increased levels of TSB leads to hyperbilirubinemia which clinically know as jaundice[6]. The elevated levels of bilirubin are also highly neurotoxicas it penetrates blood brain barrier, accumulates over there and produce kernicterus which leads to damage of brain tissue[7].

The main aim of the medical intervention is to reduce the level of elevated bilirubin and to avoid a toxic accumulation. It has been reported that approximately 10% of all new-borns needs such intervention to treat hyperbilirubinemia[8]. Currently there is two major medical intervention are available for treatment of hyperbilirubinemia i.e., exchange transfusion and photo therapy. Exchange transfusion is continuing since 1930 which causes very rapidly decrease in bilirubin label. However, this treatment is associated with complications such as metabolic acidosis, electrolyte abnormalities, hypoglycemia, hypocalcemia, volume overload and graft versus host disease, etc. Whereas, phototherapy which consists of exposure to blue light (410-460 nm) is comparatively cheap treatment intervention that has an ability to convert the bilirubin into water-soluble isomers via a photochemical reaction. This transformed configurational and structural bilirubin isomers can be excreted directly into the bile or into the urine[9,10]. A study conducted during 2003 in the United States showed that, the 4.3% of infants had TSB levels in a range in which phototherapy was recommended by the 1994 American Academy of Pediatrics (AAP) guidelines and 2.9% had values in a range in which the 1994 AAP guidelines suggest considering phototherapy[11].

Bile duct-ligated Sprague-Dawley rats are well established and widely used animal model to investigate the effect of medical intervention in elevated TSB levels[12,13]. It mimics the clinicopathological features of hyperbilirubinemia where bile duct ligation of resulted inelevated levels of both unconjugated and conjugated bilirubin in serum[14]. Research carried out over a past decade showed that both free and conjugated bilirubin effectively scavenge oxygen radicals[15,16] thus this bilirubins are classified as antioxidants[17,18]. Irradiation of bilirubin leads to the generation of vicious cycle that ends into oxidation of bilirubin[19]. Elevated levels of unconjugated bilirubin via fragmentation of bilirubin serves as reactive oxygen species *in vivo* and that leads to oxidative stress to animals[20]. These oxidative stress caused hepatic damage in the animals.

Phototherapy is widely used for lowering elevated levels of unconjugated bilirubin[10,21,22]. However, conventional phototherapy systems which used fluorescent or halogen light sources has the limitation of light intensity and portability. Hence, to overcome this limitation we have implement Light Emitting Diodes (LEDs) with blue region of visible spectrum. In the present investigation we have used edixeon S series power LED which has minimum luminous flux of 256.3 mW at 350 mA current and has narrow range of wavelength that is 440-460 nm than other LED's. The low power LED has lower luminous ranging in between 17.6-30.3 mW and also has lower spectral irradiance which provides advantages of low cost with long operational lifespans over conventional or fiberoptic phototherapy. However, its potential in the treatment of hyperbilirubinemia has not been yet evaluated. Hence, the aim of present investigation was to study the effect of LEDs on obstructive jaundice induced hyperbilirubinemia in laboratory animals.

MATERIALS AND METHODS

Animals: Adult male Wistar rats (180-220 g) were obtained from the National Institute of Biosciences, Pune, India. They were housed in cages in a facility maintained at 24±1°C, with a relative humidity of 45-55% and 12:12 h dark/light cycle (except experimental condition). The animals had free access to standard pellet chow (Pranav Agro Industries Ltd., Sangli, India) and filtered water throughout the experimental period. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved (CPCSEA/70/2012) by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. Animals were transferred to testing laboratory 1 h before the experiment for adaptation.

Chemicals: Bilirubin IX was purcahsed from Otto Chemie Pvt., Ltd., Mumbai, India. The 1,1',3,3'-tetraethoxypropane,

crystalline beef liver catalase, reduced GSH and 5,5-dithiobis (2-nitrobenzoic acid) were purchased from SD fine chemicals, Mumbai, India. Sulphanilamides, naphthylamine diamine HCl and phosphoric acid were obtained from Loba Chemi Pvt., Ltd., Mumbai, India. All other chemicals were commercial products of at least analytical grade.

Induction of obstructive jaundice and drug treatment schedule: Rats were randomly divided into six groups of six rats as follows:

Group I: **Normal control:** Rats did not undergo any surgery and did not receive any bile duct ligation. They were exposed to normal day light for 12 h for 3 days

Group II: **Sham:** Rats were undergone surgery but did not receive any bile duct ligation. They were exposed to normal day light for 12 h for 3 days

Group III: **Control:** Rats were undergone surgery and received bile duct ligation. They were exposed to normal day light for 12 h for 3 days

Group IV: **LED (Low):** Rats were undergone surgery and received bile duct ligation. They were subjected to whole body irradiation of LED with intensity 0.06 W m^{-2} for 5 h for 3 days

Group V: **LED (High):** Rats were undergone surgery and received bile duct ligation. They were subjected to whole body irradiation of LED with intensity 1.0 W m^{-2} for 5 h for 3 days

Group VI: **Per se:** Rats did not undergo any surgery however, they were subjected to whole body irradiation of LED with intensity 1.0 W m^{-2} for 5 h for 3 days

The induction of obstructive jaundice was carried out as previously described[12]. Briefly, after overnight fasting, ketamine HCl was administered (50 mg kg^{-1}, i.p.). Povidone-iodine (1%) was used for local cleansing. The peritoneal cavity was entered through a 2 cm upper midline laparotomy. In rats of groups II (sham groups) the common bile duct was freed from the surrounding soft tissue without ligation. In group III, IV and V animals (obstructive jaundice groups), the common bile duct was identified and double ligated with 5-0 silk and divided between the two ligatures. The fascia was closed with running 3-0 polyglactin and the skin was closed with continuous subcuticular 4-0 polyglactin sutures.

Irradiation procedure: The rats in the metabolic cages were subjected to whole body irradiation using LED lamps (Sylvania, Waltham, MA). The lamps had an emission maximum at 460 nm and a half-band width of 40 nm. The light fluence, at the level of the irradiated animals was 1.2 W m^{-2} as assessed with a radiometer. The fluence was periodically controlled during the photo-treatment.

After 3 days of exposure, rats fasted overnight and after 24 h, they were sequentially anesthetized with anesthetic ether for about 30-40 sec. The blood was withdrawn by the retro-orbital puncture. Each blood sample was collected into separate vials for the determination of serum parameters. After blood collection, the animals were sacrificed by cervical dislocation and then liver was removed. The specimens were divided into two portions: One portion was used for biochemical estimation and another portion was processed for histopathological examination.

Serum biochemistry: The serum was separated by centrifugation using an Eppendorf cryocentrifuge (model No. 5810, Eppendorf, Hamburg, Germany) maintained at 4°C and run at a speed of 7000 rpm for 15 min. The levels of serum albumin, alkaline phosphatase (ALP), direct bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin and gamma-glutamyl transferase (GGT) were measured by a spectrophotometer (UV-visible spectrophotometer, Jasco V-530, Tokyo, Japan) using commercially available reagent kits according to procedure provided by manufacturer (Accurex Biomedical Pvt., Ltd., Mumbai, India)[23-32].

Biochemical estimations

Preparation of tissue homogenate: For liver homogenization, tissue segments were mixed with 0.1 M phosphate buffer and homogenized on ice for 60 sec at 10000 rpm in a homogenizer (Remi Equipment Pvt., Ltd., Remi Motors Ltd., Mumbai, India). Supernatant of tissue homogenates was employed to estimate superoxide dismutase (SOD), reduced GSH, lipid peroxidation (malondialdehyde (MDA) content) and nitric oxide (NO content).

Determination of total protein, SOD, GSH, MDA and NO: The level of total protein, SOD, GSH, MDA and NO in liver and kidney homogenate was determined according to earlier reported methods[33-42].

Histopathological examination: Liver tissues were stored in 10% formalin for 24 h. The specimen was dehydrated and placed in xylene for 1 h (3 times) and later in ethyl alcohol (70, 90 and 100%) for 2 h. The infiltration and impregnation were carried out by treating with paraffin wax twice, each time for 1 h. Tissue specimens were cut into sections of 3-5 mm thickness and were stained with hematoxylin and eosin (H and E). The specimen was mounted on the slide by use of distrene phthalate xylene (DPX) as a mounting medium. Sections were examined under a light microscope for the inspection of the histopathology features of specimen and infiltration of cells. The various changes in histological features were graded as grade 0 (not present or very slight), grade 1 (mild), grade 2 (moderate) and grade 3 (severe) as described earlier[43,44].

Statistical analysis: Data were expressed as Mean±Standard Error Means (SEM). Data analysis was performed using software (v5.0, GraphPad, San Diego, CA). Data of biochemical parameters were analyzed using one-way analysis of variance (ANOVA) and followed by Tukey's multiple range tests for each parameter separately. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of LED irradiation treatment on serum albumin, alkaline phosphatase, AST, ALT and GGT in rats: The level of serum albumin, alkaline phosphatase, AST, ALT and GGT levels were significantly ($p<0.001$) increased in the control rats as compared to normal and sham rats. The LED irradiation (low and high) treatment significantly decreased serum albumin, alkaline phosphatase, AST, ALT and GGT levels ($p<0.01$ and $p<0.001$) as compared to control rats. However, serum albumin, alkaline phosphatase, AST, ALT and GGT did not differ significantly in normal, sham as well as per se treated group (Table 1).

Effect of LED irradiation treatment on total bilirubin and direct bilirubin levels in rats: Control rats showed significantly ($p<0.001$) increased levels of total bilirubin and direct bilirubin as compared to normal and sham rats. When compared with control rats, there was significant ($p<0.01$ and $p<0.001$) decrease in the levels of total bilirubin and direct bilirubin after LED (low and high) irradiation treatment. However, there was no any significant difference in the levels of total bilirubin and direct bilirubin of per se rats when compared with normal and sham control rats (Table 1).

Effect of LED irradiation treatment on hepatic SOD, GSH, MDA and NO in rats: Control rats showed significantly ($p<0.001$) decrease in the level of SOD and GSH in hepatic tissue as compared to normal and sham rats. There was significant increase ($p<0.001$) in the levels of hepatic MDA and NO in control rats as compared to normal and sham rats. Treatment with LED irradiation (low and high) showed significant elevation ($p<0.01$ and $p<0.001$) in the level of hepatic SOD and GSH as compared to control rats. Whereas hepatic MDA and NO levels were significantly decreased after LED (low and high) irradiation as compared to control rats. The level of SOD, GSH, MDA and NO in hepatic tissue did not significantly differ in per se group as compared to normal and sham group (Fig. 1).

Effect of LED irradiation treatment on pathological alteration in rat liver: In the histopathological studies, normal and sham control as well as per se animals, showed normal central vein in liver parenchymal cells and without any signs of inflammation as well as necrosis in cells (Fig. 2a, b, f). However, there was evidence of congestion and necrosis in hepatic tissue from per se group rats (grade 1). The histopathological examination of liver from control rats showed inflammatory cells (grade 4), cytoplasmic vacuolation (grade 3), centrilobular necrosis (grade 2) and vascular

Table 1: Effect of LED irradiation treatmenton albumin, alkaline phosphatase, AST, ALT, GGT, total bilirubin and direct bilirubin levels in rats

Treatments	Albumin (g dL^{-1})	Alkaline phosphatase (IU l^{-1})	AST	ALT	GGT	Total bilirubin (mg%)	Direct bilirubin (mg%)
			----------(IU l^{-1})----------				
Normal	617.9±0.12	617.9±44.09	18.51±0.05	36.57±4.98	21.05±0.85	0.44±0.03	0.30±0.02
Sham	614.1±0.23	614.1±22.52	16.86±0.2	38.64±4.52	19.58±2.41	0.39±0.05	0.29±0.03
Control	992.7±0.12$^{###,\$\$\$}$	992.7±21.05$^{###,\$\$\$}$	15.43±0.11$^{###,\$\$\$}$	99.92±5.2$^{###,\$\$\$}$	42.82±0.96$^{###,\$\$\$}$	1.30±0.04$^{###,\$\$\$}$	0.59±0.02$^{###,\$\$\$}$
LED (low)	869.9±0.06**	869.9±42.92**	14.28±0.13**	76.84±2.6**	32.50±1.92**	0.93±0.06**	0.46±0.03*
LED (high)	735.5±0.16***	735.5±26.17***	23.87±0.22***	54.55±4.75***	27±2.01***	0.68±0.07***	0.35±0.04***
Per se	670.3±0.17	670.3±20.16	14.71±0.13	41.08±3.64	21.41±2.99	0.31±0.04	0.29±0.02

Data are expressed as Mean±SEM (n = 5) and analyzed by one-way ANOVA followed by multiple range test for each parameter separately, **p<0.01 and ***p<0.001 as compared to control group, $^{###}$p<0.001 as compared to normal group and $^{\$\$\$}$p<0.001 as compared to sham group

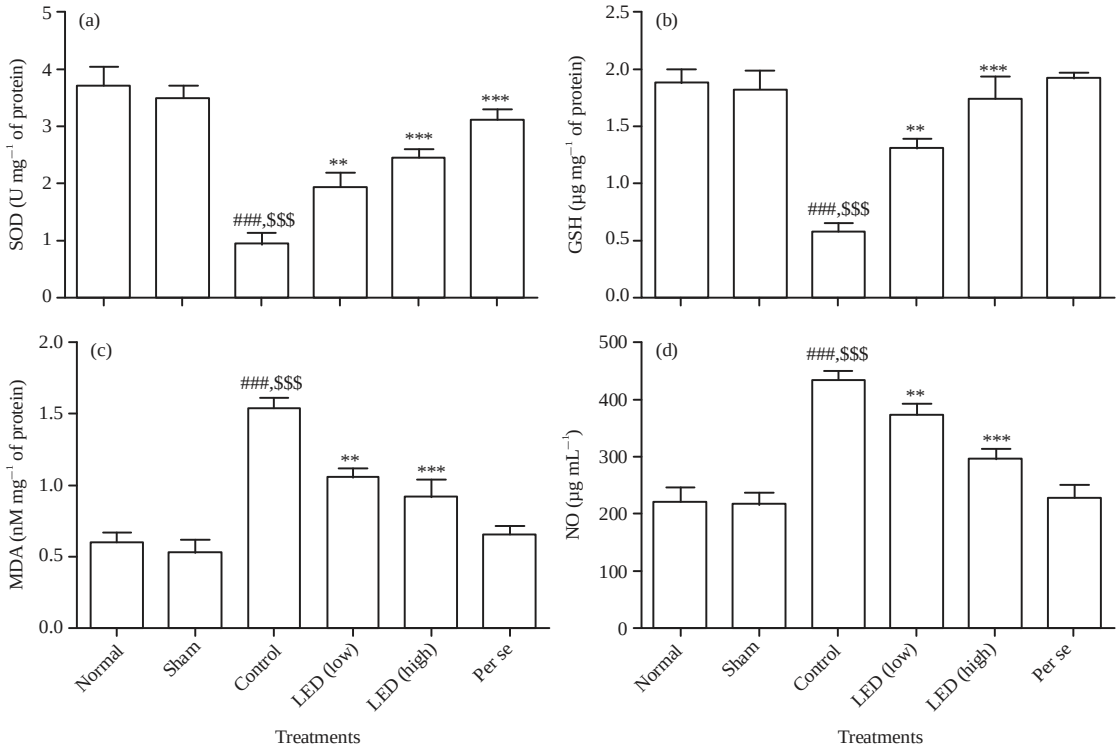

Fig. 1(a-d): Effect of LED irradiation treatmenton hepatic, (a) SOD, (b) GSH, (c) MDA and (d) NO in rats, data are expressed as Mean±SEM (n = 5) and analyzed by one-way ANOVA followed by multiple range test for each parameter separately, **p<0.01 and ***p<0.001 as compared to control group, $^{###}$p<0.001 as compared to normal group and $^{\$\$\$}$p<0.001 as compared to sham group

Table 2: Effect of LED irradiation treatmenton pathological alteration in rat liver

Treatments	Inflammatory infiltration	Plasma cells	Congestion	Edema	Vaculization	Necrosis
Normal	0	0	+	0	+	0
Sham	0	0	+	+	0	0
Control	++++	+++	++	+++	+++	++
LED (Low)	++	++	++	++	++	+
LED (High)	+	+	+	0	+	+
Per Se	0	0	+	+	+	+

0: No abnormality detected, +: Damage/active changes up to less than 25%, ++: Damage/active changes up to less than 50%, +++: Damage/active changes up to less 75% and ++++: Damage/active changes up to more than 75%

congestion (grade 2) of the hepatocytes (Fig. 2c). However, hepatic tissue from LED (low) irradiation treatment showed moderate histopathological changes in liver marked by sinusoidal congestion (grade 2), cytoplasmic vacuolation (grade 2) and the presence of inflammatory cells (grade 2) (Fig. 2d). In the LED (high) irradiation treated rats, the histology of liver showed a mild degree of vacuolization (grade 1), necrosis (grade 1) and congestion (grade 1) around central vein. It also showed evidence of moderate inflammatory cells with few plasma cells (grade 1) were present in its hepatic tissue (Fig. 2e) (Table 2).

DISCUSSION

Hyperbilirubinemia is a condition where there is a rise in the level of unconjugated bilirubin in the blood. In normal circulatory blood, Red Blood Cells (RBCs) contain the hemoglobin which is a carrier of an oxygen and this hemoglobin broken down from RBCs when it undergoes lysis, either due to normal physiology or as the result of a variety of pathologic processes. Enzyme haeme oxygenase catalyses played a vital role in the conversion of haeme to bilirubin via biliverdin. Then this unconjugated form of bilirubin bound to albumin and then in the liver it ultimately converted into its

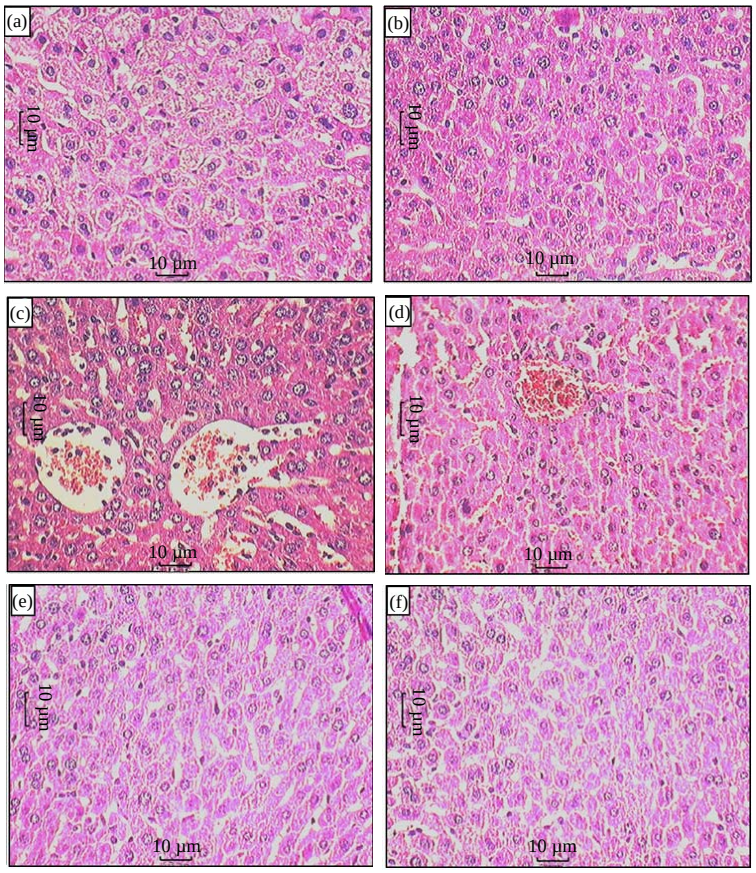

Fig. 2(a-f): Effect of LED irradiation treatmenton pathological alteration in rat liver, photomicrograph of sections of liver of (a) Normal, (b) Sham, (c) Control, (d) LED (low) treated, (e) LED (high) treated rats and (f) Per se rats, H and E staining at 40X

more water-soluble form conjugated bilirubin by the enzyme uridine diphosphoglucuronate-glucuronosyltransferase.

Conjugated bilirubin mono-glucuronide and bilirubin di-glucuronide can then be excreted into the bile, ultimately reaching the intestines for excretion[45]. In the new-born, hyperbilirubinemia resulted from an insufficiency in the metabolism this elevated unconjugated bilirubin in the liver.

Research carried out over past decades proved that phototherapy is and well established and more effective treatment strategy for this unconjugated hyperbilirubinemia[10,21,22]. Moreover, conventional phototherapy systems produced an irradiance of up to approximately 40 µW cm^{-2} nm^{-1} (KL Tan). However, the successful drop in the hyperbilirubinemia is depend upon the amount of skin exposed to phototherapy as well as the irradiance delivered by the phototherapy system[46]. The phototherapy used in the present investigation has an edixeon S series power LED with minimum luminous flux of 256.3 mW. Moreover, it a lso has narrow range of wavelength (440-460 nm) which provides a advantage high irradiance with fastest corresponding photo transformation rate by these LED's.

It has been reported that bilirubin acts as a substrate for peroxidase that converted into biliverdin[47]. Superoxides and peroxyradicals react with bilirubin resulted in elevated biliverdin level[48]. Furthermore, bilirubin ditaurate (BR-DT) react with HOCl that also causes increased biliverdin level[48]. Recent reports suggested that bilirubin has a free radical quenching ability, thus reduced the elevated levels of reactive oxygen species and served as an antioxidant[20]. However, in neonatal hyperbilirubinemia where there is an elevated level of unconjugated bilirubin which may lead to life threatening conditions like kernicterus and encephalopathy[7] in such condition, phototherapy is used for lowering these elevated levels of unconjugated bilirubin.

It has been reported that in serum, a total protein comprised 50% of albumin and correlated with each other[49].

Albumin maintains the plasma volume and transportation of various substances in the blood[50-52]. Hepatic cells injury decreased the serum protein levels might be due to denaturation of proteins with elevated extravascular loss[53]. An elevated level of total cholesterol and triglycerides is an important feature of altered fatty acid metabolism[54]. This alteration in fatty acid metabolism caused fat accumulation in hepatocytes, leading to liver damage. Changes in AST, ALT, ALP, GGT and bilirubin levels are also indicators of hepatic dysfunction[55,56]. Results from our study showed that LED irradiation treatment caused significantly deceased in these elevated levels of hepatic plasma markers. Similarly, the liver histopathological analysis also in line with these biochemical findings suggested that LED irradiation treatment played an important role in the improvement of hepatic damage via modulation of hepatic enzymes.

The SOD and GSH are the two important endogenous antioxidant enzymes present in tissue and play a vital role in detoxifying free radicals and other cytotoxic chemical species[57-64]. Depletion of t hese enzymes result from the elevated production of reactive oxygen species generation[65-73]. The MDA, an end product of lipid peroxidation which is responsible for bio-membrane damage through modulation of unsaturated bonds of fatty acids[36,74-82]. In the current study, we observed a significant decrease in the levels of SOD and GSH whereas significant increase in the MDA and NO in liver tissues reflected the elevated oxidative stress. Whereas, LED irradiation treatment showed inhibition in these elevated oxidative stress via modulation of antioxidant status of the hepatic tissue.

CONCLUSION

In the present investigation, two LED phototherapy light intensities (low and high) were produced by the array. Under phototherapy, these two LED phototherapy groups exhibited significant decreased in bilirubin levels and corrected elevated oxidative stress which reflects a strong direct correlation the between LED light intensity and the oxidative stress. Future work will explore the threshold LED light intensity needed to phototrans form bilirubin *in vitro* which will apply this system to further clinical evaluation. In conclusion, the LED irradiation treatment showed inhibition in the elevated bilirubin levels via modulation of hepatic biomarkers as well as elevated oxidative stress. The use of edixeon S series power LED may be beneficial in terms of low cost of LED emitters and power requirements which can make this LED phototherapy a useful and possibly a preferred modality for the treatment of neonatal jaundice.

ACKNOWLEDGMENTS

The researchers would like to acknowledge Dr. S.S. Kadam, Vice Chancellor and Dr. K.R. Mahadik, Principal, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, India for providing necessary facilities to carry out the study. The authors would also like to acknowledge Invaluesys Research Group to carry out statistical analysis of the study.

REFERENCES

1. Brown, A.K., K. Damus, M.H. Kim, K. King and R. Harper *et al*., 1999. Factors relating to readmission of term and near term neonates in the first two weeks of life. J. Perinat. Med., 27: 263-275.
2. Ford, J.B., C.S. Algert, J.M. Morris and C.L. Roberts, 2012. Decreasing length of maternal hospital stay is not associated with increased readmission rates. Aust. N. Z. J. Public Health, 36: 430-434.
3. Lee, K.S. and M. Perlman, 1996. The impact of early obstetric discharge on newborn health care. Curr. Opin. Pediatr., 8: 96-101.
4. Lee, K.S., M. Perlman, M. Ballantyne, I. Elliott and T. To, 1995. Association between duration of neonatal hospital stay and readmission rate. J. Pediatr., 127: 758-766.
5. Woodgate, P. and L.A. Jardine, 2015. Neonatal jaundice: Phototherapy. BMJ Clin. Evidence.
6. Woodall, D. and J.G. Karas, 1992. A new light on jaundice. A pilot study. Clin. Pediatr. (Phila), 31: 353-356.
7. Thyagarajan, B. and S.S. Deshpande, 2014. Cotrimoxazole and neonatal kernicterus: A review. Drug Chem. Toxicol., 37: 121-129.
8. Schuman, A.J. and G. Karush, 1992. Fiberoptic vs conventional home phototherapy for neonatal hyperbilirubinemia. Clin. Pediatr., 31: 345-352.
9. Bhutani, V.K., 2011. Phototherapy to prevent severe neonatal hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatric, 128: 1046-1052.
10. Seidman, D.S., J. Moise, Z. Ergaz, A. Laor, H.J. Vreman, D.K. Stevenson and R. Gale, 2000. A new blue light-emitting phototherapy device: A prospective randomized controlled study. J. Pediatr., 136: 771-774.
11. Atkinson, L.R., G.J. Escobar, J.I. Takayama and T.B. Newman, 2003. Phototherapy use in jaundiced newborns in a large managed care organization: Do clinicians adhere to the guideline? Pediatrics, 111: e555-e561.
12. Sen, M., A. Inan, S. Yenidunya, M. Ergin and C. Dener, 2006. Effect of vitamin A on the CD44 expression in the small intestine of rats with obstructive jaundice. Eur. Surg. Re., 38: 347-352.

13. Kandhare, A.D., K.S. Raygude, P. Ghosh, T.P. Gosavi and S.L. Bodhankar, 2011. Patentability of animal models: India and the globe. Int. J. Pharm. Biol. Arch., 2: 1024-1032.
14. Pettenazzo, A., E. Reddi, B. Granati, S. Camurri, P. Zaramella and F. Rubaltelli, 1985. Cholestasis induced in Gunn rats as an experimental model of bronze baby syndrome. Primary Photo-Processes Biol. Med., 85: 421-421.
15. Ostrow, J.D. and R.V. Branham, 1970. Photodecomposition of bilirubin and biliverdin *in vitro*. Gastroenterology, 58: 15-25.
16. McDonagh, A.F., 1971. The role of singlet oxygen in bilirubin photo-oxidation. Biochem. Biophys. Res. Commun., 44: 1306-1311.
17. Stocker, R. and E. Peterhans, 1989. Antioxidant properties of conjugated bilirubin and biliverdin: Biologically relevant scavenging of hypochlorous acid. Free Radic. Res. Commun., 6: 57-66.
18. Jirsa, Jr. M., M. Sip and M. Jirsa, 1990. Influence of hyperbaric oxygenation on bilirubin and ditaurobilirubin auto-oxidation and porphyrin-sensitized photo-oxidation. J. Photochem. Photobiol. B: Biol., 5: 295-302.
19. Pedersen, A.O., F. Schonheyder and R. Brodersen, 1977. Photooxidation of human serum albumin and its complex with bilirubin. Eur. J. Biochem., 72: 213-221.
20. Yamaguchi, T., F. Horio, T. Hashizume, M. Tanaka, S. Ikeda, A. Kakinuma and H. Nakajima, 1995. Bilirubin is oxidized in rats treated with endotoxin and acts as a physiological antioxidant synergistically with ascorbic acid *in vivo*. Biochem. Biophys. Res. Commun., 214: 11-19.
21. Vreman, H.J., R.J. Wong, D.K. Stevenson, R.K. Route and S.D. Reader *et al*., 1998. Light-emitting Diodes: A novel light source for phototherapy. Pediatr. Res., 44: 804-809.
22. Rosen, D. and A. Rosen, 2000. Therapeutic method and internally illuminated garment for the management of disorders treatable by phototherapy. Patent US6045575. http://www.google.ch/patents/US6045575.
23. Adil, M., A.D. Kandhare, G. Dalvi, P. Ghosh, S. Venkata, K.S. Raygude and S.L. Bodhankar, 2016. Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. Ren. Fail, 38: 996-1006.
24. Adil, M., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2016. Sodium arsenite-induced myocardial bruise in rats: Ameliorative effect of naringin via TGF-β/Smad and Nrf/HO pathways. Chem. Biol. Interact., 253: 66-77.
25. Devkar, S., A. Kandhare, A. Zanwar, S. Jagtap, S. Katyare, S. Bodhankar and M. Hegde, 2016. Hepatoprotective effect of withanolide rich fraction in acetaminophen intoxicated rat: Decisive role of TNF-α, IL-1β, COX-II and iNOS. Pharmaceut. Biol., 54: 2394-2403.
26. Honmore, V.S., A.D. Kandhare, P.P. Kadam, V.M. Khedkar and D. Sarkar *et al*., 2016. Isolates of *Alpinia officinarum* Hance as COX-2 inhibitors: Evidence from anti-inflammatory, antioxidant and molecular docking studies. Int. Immunopharmacol., 33: 8-17.
27. Kamble, H., A.D. Kandhare, S. Bodhankar, V. Mohan and P. Thakurdesai, 2013. Effect of low molecular weight galactomannans from fenugreek seeds on animal models of diabetes mellitus. Biomed. Aging Pathol., 3: 145-151.
28. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Prophylactic efficacy and possible mechanisms of oligosaccharides based standardized fenugreek seed extract on high-fat diet-induced insulin resistance in C57BL/6 mice. J. Applied Pharma. Sci., 5: 35-45.
29. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fibrosis in rats. Proceedings of the 24th World Allergy Congress, October 14-17, 2015, Seoul, South Korea.
30. Patil, A., A. Guru, A. Mukhrjee, A. Sengupta and S. Sarkar *et al*., 2015. Elucidation of gastro-protective activity of Morin in pylorus ligation induced gastric ulcer via modulation of oxidative stress. Der Pharmacia Lettre, 7: 131-139.
31. Saraswathi, K.Y., A. Muthal, A. Kandhare, S. Rojatkar and S. Bodhankar, 2014. Study of methanolic extract of *Artemisia pallens* wall on endurance of laboratory animals. Pharmacologia, 5: 298-309.
32. Sarkar, S., A. Sengupta, A. Mukhrjee, A. Guru, A. Patil, A.D. Kandhare and S.L. Bodhankar, 2015. Antiulcer potential of morin in acetic acid-induced gastric ulcer via modulation of endogenous biomarkers in laboratory animals. Pharmacologia, 6: 273-281.
33. Gosavi, T.P., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2012. Anticonvulsant activity of *Argentum metallicum*, a homeopathic preparation. Der Pharmacia Lettre, 4: 626-637.
34. Kandhare, A.D., K.S. Raygude, P. Ghosh and S.L. Bodhankar, 2011. The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats. Int. J. Green Pharm., 5: 236-243.
35. Kandhare, A.D., S.L. Bodhankar, V. Singh, V. Mohan and P.A. Thakurdesai, 2013. Anti-asthmatic effects of type-A procyanidine polyphenols from cinnamon bark in ovalbumin-induced airway hyperresponsiveness in laboratory animals. Biomed. Aging Pathol., 3: 23-30.
36. Kandhare, A.D., P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2013. Elucidation of molecular mechanism involved in neuroprotective effect of Coenzyme Q10 in alcohol-induced neuropathic pain. Fundam. Clin. Pharmacol., 27: 603-622.
37. Aswar, U.M., A.D. Kandhare, V. Mohan and P.A. Thakurdesai, 2015. Anti-allergic effect of intranasal administration of type-a procyanidin polyphenols based standardized extract of cinnamon bark in ovalbumin sensitized BALB/c mice. Phytother. Res., 29: 423-433.
38. Mukherjee, A., A.D. Kandhare and S.L. Bodhankar, 2015. Effect of chrysin on gentamicin-induced nephrotoxicity in laboratory animals. Pharmacologia, 7: 296-307.

39. Sarkate, A.P., P.R. Murumkar, D.K. Lokwani, A.D. Kandhare, S.L. Bodhankar, D.B. Shinde and K.G. Bothara, 2015. Design of selective TACE inhibitors using molecular docking studies: Synthesis and preliminary evaluation of anti-inflammatory and TACE inhibitory activity. SAR QSAR Environ. Res., 26: 905-923.
40. Bhilare, V.N., S.S. Dhaneshwar, A.J. Sinha, A.D. Kandhare and S.L. Bodhankar, 2016. Novel thioester prodrug of N-acetylcysteine for odor masking and bioavailability enhancement. Curr. Drug Deliv., 13: 611-620.
41. Visnagri, A., M. Adil, A.D. Kandhare and S.L. Bodhankar, 2015. Effect of naringin on hemodynamic changes and left ventricular function in renal artery occluded renovascular hypertension in rats. J. Pharmacy Bioallied Sci., 7: 121-127.
42. Visnagri, A., A.D. Kandhare and S.L. Bodhankar, 2015. Renoprotective effect of berberine via intonation on apoptosis and mitochondrial-dependent pathway in renal ischemia reperfusion-induced mutilation. Renal Fail., 37: 482-493.
43. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Acute and repeated doses (28 days) oral toxicity study of glycosides based standardized fenugreek seed extract in laboratory mice. Regul. Toxicol. Pharmacol., 72: 323-334.
44. Adil, M., A.D. Kandhare, A. Visnagri and S.L. Bodhankar, 2015. Naringin ameliorates sodium arsenite-induced renal and hepatic toxicity in rats: Decisive role of KIM-1, Caspase-3, TGF-β and TNF-α. Renal Failure, 37: 1396-1407.
45. Martin, R.J., A.A. Fanaroff and M.C. Walsh, 2014. Fanaroff and Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant. Elsevier Health Sciences, Makati, Philippines,.
46. Bertini, G., S. Perugi, S. Elia, S. Pratesi, C. Dani and F.F. Rubaltelli, 2008. Transepidermal water loss and cerebral hemodynamics in preterm infants: Conventional versus LED phototherapy. Eur. J. Pediatr., 167: 37-42.
47. Brodersen, R. and P. Bartels, 1969. Enzymatic oxidation of bilirubin. Eur. J. Biochem., 10: 468-473.
48. Stocker, R., A.N. Glazer and B.N. Ames, 1987. Antioxidant activity of albumin-bound bilirubin. Proc. Natl. Acad. Sci., 84: 5918-5922.
49. Doweiko, J.P. and D.J. Nompleggi, 1991. Reviews: Role of albumin in human physiology and pathophysiology. J. Parenteral Enteral Nutr., 15: 207-211.
50. Kandhare, A., S. Bodhankar, V. Mohan and P. Thakurdesai, 2013. Low molecular weight galactomannans from fenugreek seeds ameliorates high-fat diet-induced insulin resistance in C57BL/6 mice model. Indian J. Pharmacol., 45: S15-S15.
51. Ketkar, S., A. Rathore, A. Kandhare, S. Lohidasan, S. Bodhankar, A. Paradkar and K. Mahadik, 2015. Alleviating exercise-induced muscular stress using neat and processed bee pollen: Oxidative markers, mitochondrial enzymes and myostatin expression in rats. Integr. Med. Res., 4: 147-160.
52. Visnagri, A., A.D. Kandhare, V.S. Kumar, A.R. Rajmane and A. Mohammad *et al*., 2012. Elucidation of ameliorative effect of co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. Biomed. Aging Pathol., 2: 157-172.
53. Henry, J.B., 1996. Clinical Diagnosis and Management by Laboratory Methods. 19th Edn., Saunders, Philadelphia, 1016.
54. Muntner, P., J. Coresh, J.C. Smith, J. Eckfeldt and M.J. Klag, 2000. Plasma lipids and risk of developing renal dysfunction: The atherosclerosis risk in communities study. Kidney Int., 58: 293-301.
55. Mehta, A.K., N. Arora, S.N. Gaur and B.P. Singh, 2009. Acute toxicity assessment of choline by inhalation, intraperitoneal and oral routes in Balb/c mice. Regul. Toxicol. Pharmacol., 54: 282-286.
56. Adil, M., A.D. Kandhare, P. Ghosh, S. Venkata, K.S. Raygude ahnd S.L. Bodhankar, 2016. Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: Role of FXR and KIM-1. Renal Failure, 38: 1007-1020.
57. Adil, M., A. Visnagri, V.S. Kumar, A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2014. Protective effect of naringin on sodium arsenite induced testicular toxicity via modulation of biochemical perturbations in experimental rats. Pharmacologia, 5: 222-234.
58. Ghule, A.E., A.D. Kandhare, S.S. Jadhav, A.A. Zanwar and S.L. Bodhankar, 2015. Omega-3-fatty acid adds to the protective effect of flax lignan concentrate in pressure overload-induced myocardial hypertrophy in rats via modulation of oxidative stress and apoptosis. Int. Immunopharmacol., 28: 751-763.
59. Yin, J., W. Ren, G. Yang, J. Duan and X. Huang *et al*., 2016. L-cysteine metabolism and its nutritional implications. Mol. Nutr. Food Res., 60: 134-146.
60. Raygude, K.S., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2012. Anticonvulsant effect of fisetin by modulation of endogenous biomarkers. Biomed. Preventive Nutr., 2: 215-222.
61. Raygude, K.S., A.D. Kandhare, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. Inflammopharmacology, 20: 331-341.
62. Visnagri, A., A.D. Kandhare, S. Chakravarty, P. Ghosh and S.L. Bodhankar, 2014. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. Pharmaceut. Biol., 52: 814-828.
63. Visnagri, A., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2013. Endothelin receptor blocker bosentan inhibits hypertensive cardiac fibrosis in pressure overload-induced cardiac hypertrophy in rats. Cardiovasc. Endocrinol., 2: 85-97.

64. Kandhare, A.D., J. Alam, M.V.K. Patil, A. Sinha and S.L. Bodhankar, 2016. Wound healing potential of naringin ointment formulation via regulating the expression of inflammatory, apoptotic and growth mediators in experimental rats. Pharmaceut. Biol., 54: 419-432.
65. Goswami, S., A. Kandhare, A.A. Zanwar, M.V. Hegde and S.L. Bodhankar *et al*., 2016. Oral L-glutamine administration attenuated cutaneous wound healing in Wistar rats. Int. Wound J., 13: 116-124.
66. Honmore, V., A. Kandhare, A.A. Zanwar, S. Rojatkar, S. Bodhankar and A. Natu, 2015. *Artemisia pallens* alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers. Pharmaceut. Biol., 53: 571-581.
67. Badole, S.L., S.M. Chaudhari, G.B. Jangam, A.D. Kandhare and S.L. Bodhankar, 2015. Cardioprotective activity of *Pongamia pinnata* in streptozotocin-nicotinamide induced diabetic rats. BioMed Res. Int. 10.1155/2015/403291
68. Yin, J., J. Duan, Z. Cui, W. Ren, T. Li and Y. Yin, 2015. Hydrogen peroxide-induced oxidative stress activates NF-κB and Nrf2/Keap1 signals and triggers autophagy in piglets. RSC Adv., 5: 15479-15486.
69. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fibrosis in rats: Decisive role of Bax, Nrf2, NF-κB, Muc5ac, TNF-α and IL-1β. Chemico-Biol. Interact., 237: 151-165.
70. Kumar, V.S., A.R. Rajmane, M. Adil, A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2014. Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. J. Biomed. Res., 28: 132-145.
71. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2016. Glycosides based standardized fenugreek seed extract ameliorates bleomycin-induced liver fibrosis in rats via modulation of endogenous enzymes. J. Pharm. Bioall. Sci., (In Press).
72. Kandhare, A.D., A. Patil, A. Guru, A. Mukherjee and A. Sarkar *et al*., 2015. Ameliorative effect of ferulic acid against acetic acid induced ulcerative colitis: Role of HO-1 and Nrf2. Pharmacologia, 7: 114-124.
73. Kandhare, A.D., M.V. Patil and S.L. Bodhankar, 2015. L-arginine attenuates the ethylene glycol induced urolithiasis in ininephrectomized hypertensive rats: Role of KIM-1, NGAL and NOs. Renal Fail., 37: 709-721.
74. Kandhare, A.D., P. Ghosh and S.L. Bodhankar, 2014. Naringin, a flavanone glycoside, promotes angiogenesis and inhibits endothelial apoptosis through modulation of inflammatory and growth factor expression in diabetic foot ulcer in rats. Chemico-Biol. Interact., 219: 101-112.
75. Kandhare, A.D., P. Ghosh, A.E. Ghule, G.N. Zambare and S.L. Bodhankar, 2013. Protective effect of *Phyllanthus amarus* by modulation of endogenous biomarkers and DNA damage in acetic acid induced ulcerative colitis: Role of phyllanthin and hypophyllanthin. Apollo Med., 10: 87-97.
76. Kandhare, A.D., V.S. Kumar, M. Adil, A.R. Rajmane, P. Ghosh and S.L. Bodhankar, 2012. Investigation of gastro protective activity of *Xanthium strumarium* L. by modulation of cellular and biochemical marker. Orient. Pharmacy Exp. Med., 12: 287-299.
77. Mohod, S.M., A.D. Kandhare and S.L. Bodhankar, 2016. Gastroprotective potential of pentahydroxy flavone isolated from *Madhuca indica* J. F. Gmel. Leaves against acetic acid-induced ulcer in rats: The role of oxido-inflammatory and prostaglandins markers. J. Ethnopharmacol., 182: 150-159.
78. Kandhare, A.D., M.V.K. Patil and S.L. Bodhankar, 2016. Ameliorative effect of alkaloidal fraction of leaves of *Alstonia scholaris* against acetic acid induced colitis via modulation of oxido-nitrosative and pro-inflammatory cytokines. Pharmacologia, 7: 170-181.
79. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitoterapia, 83: 650-659.
80. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Therapeutic role of curcumin in prevention of biochemical and behavioral aberration induced by alcoholic neuropathy in laboratory animals. Neurosci. Lett., 511: 18-22.
81. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule, T.P. Gosavi, S.L. Badole and S.L. Bodhankar, 2012. Effect of hydroalcoholic extract of *Hibiscus rosa sinensis* Linn. leaves in experimental colitis in rats. Asian Pac. J. Trop. Biomed., 2: 337-344.
82. Kandhare, A.D., K.S. Raygude, V.S. Kumar, A.R. Rajmane and A. Visnagri *et al*., 2012. Ameliorative effects quercetin against impaired motor nerve function, inflammatory mediators and apoptosis in neonatal streptozotocin-induced diabetic neuropathy in rats. Biomed. Aging Pathol., 2: 173-186.