IJPSR (2016), Vol. 7, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 September, 2015; received in revised form, 07 November, 2015; accepted, 17 December, 2015; published 01 March, 2016

PROTECTION OF LOW LET RADIATION-INDUCED DNA DAMAGE IN RAT BONE MARROW CELLS BY FREE RADICAL SCAVENGER CURCUMIN

Paonam Priyobrata Singh *, Khoirom Ratipiyari Devi, Moirangthem Medhapati Devi, Dhanaraj Singh Thokchom and Gurumayum Jitendra Sharma

Redox Biology Laboratory, Centre of Advanced Study in Life Sciences, Manipur University, Imphal-795003, India.

Keywords:

Hydroxyl radical scavenging activity, Super oxide radical scavenging activity, Reducing power, Comet assay, pBR 322 DNA

Correspondence to Author:
Paonam Priyobrata Singh

Ph. D fellow
Redox Biology Laboratory,
Centre of Advanced Study in Life Sciences, Manipur University,
Imphal 795003, India.

E-mail: priyopaonam@gmail.com

ABSTRACT: Antioxidant potentials and radioprotective properties of curcumin were studied. Free radical scavenging activity of curcumin was measured spectrophotometrically using ferric ion reducing power assay, DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical test, hydroxyl radical scavenging activity assay, nitric oxide scavenging assay and superoxide scavenging capacity. The antioxidant properties depended upon concentration of curcumin in the reaction mixture. Curcumin exhibited good protection against low LET radiation-induced DNA damage. Treatment of ethanolic solutions of curcumin before irradiation significantly reduced comet tail DNA and length ($P < 0.05$.) in bone marrow cells of rat as revealed by alkaline single cell gel electrophoresis. Treatment of curcumin solutions to plasmid (pBR322) DNA prior to exposure of radiation under *in vitro* condition protect the DNA damage as indicated by significant reduction of the gamma radiation-induced conversion of CCC (covalently closed circular) to OC (open circular) form. The findings indicate that curcumin possesses promising antioxidant and radioprotective properties, and hence, has an important role in radiation countermeasures.

INTRODUCTION: Curcumin (diferuloyl methane) is an orange yellow coloured hydrophobic polyphenolic compound (**Fig.1**) occurring as a major pigment of turmeric (*Curcuma longa* Linn.). It can exist in keto and enol tautomeric forms, in which the enol form is more energetically stable in the solid phase and in solution [1]. This dietary antioxidant is widely used as colouring and flavouring agent in food and has been known to exhibit diverse biological functions such as choleretic, cytotoxic, anti-oxidative, anti-amyloid, anti-hepatotoxic, anti-inflammatory, anti-fungal, anti-bacterial, anti-tumour and anti-rheumatic properties [2, 3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(3).1168-78

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (3).1168-78

This natural product has also shown promising role in treating many other diseased conditions including obesity, kidney and liver disease, eye and lung disorders, allergies, pancreatitis and more [5, 6]. Reports show that curcumin can scavenge oxygen free radicals and inhibit lipid peroxidation [7]. Curcumin and curcuminoids have been shown to possess significant free radical scavenging activities as have been revealed by DPPH, superoxide and NO scavenging and TRAP and FRAP assays [8]. It has been used as dietary supplement as well as therapeutic agent in Chinese and other Asian medicines for centuries [9]. Moreover, curcumin has been considered extremely safe even at very high doses through studies using various animal models and in human studies [10].

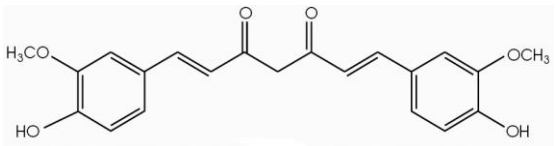

FIG. 1: STRUCTURE OF CURCUMIN

Reactive oxygen species (ROS) are free radicals which are the atoms of oxygen having central unpaired electron produced by environmental agents like sunlight, ultraviolet light, ionizing radiation, chemical reactions or normal metabolic processes in our body. ROS such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide, peroxynitrite radicals, etc. play important role in oxidative stress resulting to various chronic human disorders [11]. Oxidative stress associated with free radicals contributes to more than hundred disorders in humans including atherosclerosis, rheumatoid arthritis, diabetes mellitus, stroke, myocardial infarction, hypertension, reperfusion injury, Alzheimer diseases, gastritis, Parkinson's disease, haemorrhagic shock, coronary heart diseases, neuro-degeneration, cataract, carcinogenesis, inflammatory disorders, AIDS as well as age-related brain disorders [12, 13].

Antioxidants are extremely useful chemical substances capable of either prevent or quench free radical reactions and delay or inhibit the oxidation of cellular molecules [14]. They can effectively neutralize free radicals and oxygen-derived species which become companions to their unpaired electrons by interfering with oxidation process, chelating catalytic metals and also by acting as oxygen scavengers [15]. Thus, antioxidants act as major defence molecules against radical-mediated toxicity by protecting the cellular entities. In healthy individuals, the production of free radicals is balanced by the anti-oxidative defence system. However, oxidative stress is generated when free radicals are generated as a result of a depletion of antioxidant levels. In recent decades, phytochemical constituents have received much attention due to their potential utilization in nutraceutical and drug industries. Antioxidants of plant origin could have great importance as therapeutic agents in aging process and free radical mediated diseases. The present study aims at determining anti-oxidative potentials of curcumin and its protection against radiation-induced DNA damage using *in vitro* and *in vivo* models.

MATERIALS AND METHODS:

Chemicals and Reagents:

Tris base, high melting agarose, low melting point agarose, triton X-100, DMSO, ascorbic acid, curcumin, TCA, DPPH, glutathione, hypoxanthine, quercetin and xanthine oxidase were obtained from Sigma-Aldrich, USA. $FeSO_4$, sodium salicylate, Na_2-EDTA, HBSS, nitroblue tetrazolium (NBT) and frosted microscope slides were procured from HiMedia, India. Plasmid pBR322 DNA and ethidium bromide were purchased from Bangalore Genei, India). Other chemicals and reagents were of analytical grade obtained from Merck, India.

Iron (III) to Iron (II) Reducing Power Assay:

The ability of curcumin to reduce iron (III) was assessed by the method described earlier by Oyaizu [16]. Various concentration of methanolic solution of curcumin (0.75 mL) were mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%,w/v). After 30 min incubation at 50°C in water bath, 0.75 mL of trichloroacetic acid (TCA) solution (10%) was added and then centrifuged at 3000 rpm for 10 min. 1.5 mL of the upper-layer solution was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance of the resulting solution was measured at 700 nm as reducing power. Higher absorbance of the reaction mixture indicated greater reducing power. The mean absorbance values were plotted against concentration and a linear regression analysis was carried out.

DPPH Radical Scavenging Assay:

Curcumin was tested for the scavenging effect on DPPH radical according to the method of Miliauskas *et al*. [17]. Hydrogen atom or electron-donation ability of the curcumin was measured from the bleaching of the purple-coloured methanol solution of DPPH radical, which have an absorption maximum at 515 nm and disappears with reduction by an antioxidant compound. Briefly, curcumin solutions made in methanol at different concentration were added to the 100 μM solution of DPPH and incubate for 30 min at dark. The scavenging activity of the DPPH radical was determined by measuring the optical density at 515 nm using a UV–Visible spectrophotometer. The DPPH radical scavenging activity was measured through percentage decoloration using the following equation: DPPH decoloration = $((A_{515\ control} - A_{515\ sample}) / A_{515control}) \times 100$, where, A_{515}

$_{control}$ is the absorbance of the control (containing all reagents except the curcumin solution) and A $_{515\ sample}$ is the absorbance of the test sample.

Hydroxyl Radical Scavenging Assay:

Hydroxyl radical scavenging activity of curcumin solution was assessed by the method of Smirnoff and Cumbes [18]. The reaction mixture contained 1.0 mL of 1.5 mM $FeSO_4$, 0.7 mL of 6 mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate and varied concentration of curcumin. The total volume was adjusted to 3.0 mL. After incubation for 1 hour at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The scavenging activity of hydroxyl radical effect was calculated as: (1- (A_1-A_2) / A_0) x 100, where, A_0 was absorbance of the control (without curcumin), A_1 was the absorbance in the presence of curcumin and A_2 was the absorbance without sodium salicylate.

Nitric Oxide Scavenging Activity Assay:

Determination of nitric oxide radical scavenging activity was done according to the method reported by Garrat [19] with minor modifications. 0.5 mL of methanolic solutions of curcumin at different concentration was mixed with 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4). After incubation of the reaction mixture at 25^{o}C for 150 min, 0.5 mL was taken out from the reaction mixture and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and kept at room temperature for 5 min. Finally, 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. Absorbance was measured at 540 nm with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated. The nitric oxide radicals scavenging activity was calculated according to the following equation: Inhibition (%) = (A_0-A_1/ A_0) x 100, where, A_0 was the absorbance of the control (blank) and A_1 was the absorbance in the presence of the curcumin solution.

Superoxide Scavenging Assay:

Superoxide radicals generated by the hypoxanthine/xanthine oxidase system was determined spectrophotometrically by monitoring the product of nitroblue tetrazolium (NBT). Ability of the curcumin to scavenge superoxide radicals was determined from its abilities to decolorize the NBT complex [20]. The reaction mixture contained 500 μM Na_2EDTA (pH 7.4), 1.5 mM NBT, 500 μM hypoxanthine in 50 mM NaOH, 0.015 unit per mL xanthine oxidase and various concentration of curcumin solution made in methanol or KH_2PO_4-KOH buffer (as control).

Final volume was adjusted with 50 mM KH_2PO_4-KOH buffer (pH 7.4) and incubated for 30 min at 25°C. Absorbance was measured at 560 nm against a blank. Results were expressed as percentage of inhibition relative to the control, given by: Inhibition (%) = (A_{560} control − A_{560} sample/A_{560} control) × 100, where, A_{560} control was the absorbance of control reaction and A_{560} sample was the absorbance of the reaction with curcumin solution. The concentration of curcumin providing 50% inhibition (IC_{50}) was determined from the plot of inhibition percentage.

Evaluation of Radiation-Induced DNA Damage and Protection:

Experiment on radiation-induced DNA damage and protection was carried out as described in the reference [21]. The plasmid pBR322 DNA (300 ng) was exposed to 5 and 10 Gy at a dose-rate of 0.062 Gy/sec in the absence and presence of curcumin at different concentration (10-40 μg/ mL) made in 40% ethanol. Unexposed DNA solution without curcumin was taken as control. After irradiation, the DNA was electrophoresed in 1% agarose gel using 0.8 mM Tris acetic acid/2 mM EDTA buffer, pH 8 at constant voltage. The DNA bands stained with ethidium bromide were photographed and analysed by using a VilberLourmat Gel Documentation Unit. DNA bands were analysed by PerkinElmer-GeneTool Software, Version-3.07. The experiments were conducted in three replicates.

Alkaline Single-Cell Gel Electrophoresis (Comet Assay):

Preparation of Animals:

Albino rat (*Rattus norvegicus*, 2n = 42) was used as *in vivo* model with prior approval of Institutional Animal Ethics Committee (MU/LSD/HOD/BSD/09 dated November 8, 2011). Animals aged 2-3 month old and weighing about 130-180 g were housed in

sanitized rice-husk bedded polypropylene metabolic cages and maintained under standard conditions of temperature (25 ± 2°C) and humidity with 12 hours photoperiod in a 24 hour cycle. They were given with standard feed and allowed to free access of water. All the experiments were conducted strictly adhering to the guidelines prescribed by the Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India.

Dose Administration of Curcumin and Radiation Exposure:

60% ethanolic solutions of curcumin at a dose of 250 mg/kg body weight were used for oral administration to experimental animals before 24 hrs. of radiation exposure. The rats were divided into 12 different groups of 5 animals each weighing 130-180g. The control group was neither given the curcumin solution nor irradiated. Another group of animals was administered only curcumin but not exposed to γ-radiation. ^{60}Cobalt Gamma Irradiator GC-4000A (BARC, Mumbai) in the Radiation Centre of Manipur University at a dose rate of 5.3 Gy/min was used for irradiation purposes. Different doses of radiation were used depending upon the systems exposed. Animals were irradiated to γ-radiation at different doses (1, 2 and 4 Gy) corresponding to different treatments (control, 60% ethanol and curcumin solution).

Cell Sample Collection:

Animals were euthanized by cervical dislocation at 3 days post irradiation. Bone marrow cells were collected by perfusing the femur with 1 mL of cold mincing HBSS solution (with 20 mM EDTA and 10% DMSO) into a micro centrifuge tube and used immediately for comet analysis.

Comet Analysis:

Cellular DNA damages in the bone marrow cells were measured by alkaline single cell gel electrophoresis based on the original description as described by (Singh *et al*) [22] with minor modifications. Normal melting agarose (1.0% in distilled water) was layered on frosted slides previously cleaned in 99% ethanol and allowed to dry at 60˚C overnight to ensure firm adhesion. 100 µL of low-melting point agarose at 37°C was gently mixed with 100 µL of bone marrow cell suspension and layered on the pre-coated slides. The slides were cooled on ice for 15 min. Cover slips were then removed and the slides were incubated in pre-chilled lysis solution containing 2.5M NaCl, 100 mM Na_2EDTA, 10mM Tris (p^H 10) with freshly added 1% Triton X-100 and 1% DMSO at 4°C overnight. The slides were immersed in a horizontal electrophoresis tank containing ice cold alkaline buffer consisting of 300 mM NaOH and 200 mM Na_2EDTA (pH > 13.0) for 20 min. Electrophoresis was carried out in the same buffer for 30 min at 300 mA and 25 V. After electrophoresis, the slides were transferred to neutralisation buffer (0.4 M Tris-HCl, pH 7.5). The cells were dried for 10 min and stained with 50µL of 20µg/mL ethidium bromide and covered with cover slips immediately before scoring. All steps were conducted under dimmed light to prevent further DNA damage.

Image Analysis:

The slides were analysed at 400X magnification using a fluorescence microscope (Leica DM 2500) fitted with COHU high performance CCD Camera and using the Komet 5.55 (Kinetic Imaging Ltd, Liverpool) image analysis system. Two slides were observed per treatment group for 3 replicates and a total of 300 random cells were scored. Comet parameters were measured and quantization of DNA strand breaks of the stored images was done using the imaging software CASP. The tail DNA and tail length were calculated as a measure of DNA damage. The reduction of radiation-induced damage of DNA after treatment with curcumin expressed as percentage reduction in tail DNA and tail length was calculated [23,24] using the formula : Reduction (%) = (mean score in A – mean score in B) / (mean score in A – mean score in C) X 100, where A is the group of cells exposed to gamma radiation; B is the group of cells treated with curcumin and exposed to gamma radiation and C is the negative control without any radiation exposure.

Statistical Significance:

The results were expressed as mean ± SE. Analysis of variance and significant differences among the means were tested by the one-way ANOVA, using SPSS (Version 16.0 for Windows, SPSS Inc.,

Chicago, IL). Values of $P < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION:

Antioxidant Activities:

Iron (III) to Iron (II) Reducing Power:

Reducing power is a significant indicator and supporting feature of antioxidant activity. This assay is based on the reduction of Fe^{3+} to Fe^{2+} in which the yellow colour of the test solution changes to various shades of blue, depending on the reducing power of the sample. The reducing ability of a compound generally depends on the existence of antioxidants which exert the antioxidant activity by breaking the free radical chain through donation of a hydrogen atom. In the presence of reducing agent, conversion of Fe^{3+}/ferricyanide complex to ferrous form takes place.

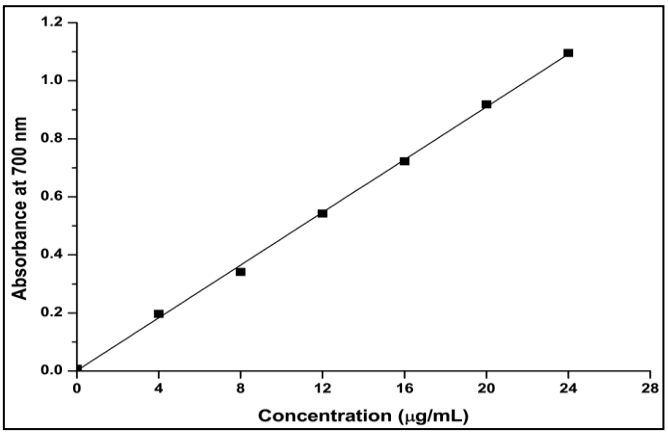

FIG. 2: FERRIC ION REDUCING CURVE AS INDICATED BY ITS RATE OF INCREASE OF ABSORBANCE.

Increasing absorbance at 700 nm indicates an increase in reductive ability. **Fig. 2** presents the reductive capabilities of curcumin solution. As per the graph obtained, it is clear that the reducing power of curcumin increases with increase in concentration.

DPPH Radical Scavenging Assay:

Several methods have been developed to study the free radical scavenging capacity and antioxidant potentials of plant-derived compounds. DPPH assay is well accepted for its ability of a test compound to act as free radical scavenger by determining the disappearance of the free radical using a spectrophotometer. The method has been used frequently to determine scavenging activity of natural compounds because of its easy, sensitive and relatively short duration required for analysis. DPPH is a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise. It accepts an electron or hydrogen radical to become a stable molecule. Methanolic solution of DPPH gives rise to the deep violet colour characterised by an absorption maxima centred at around 515 nm which decreases with the scavenging of proton radical. Methanolic solution of curcumin exhibits a good potential to act as a free radical scavenger (**Fig. 3**). A concentration dependent decrease in the absorbance of DPPH was observed with increasing concentration of curcumin giving an IC_{50} of 3.92µg/mL.

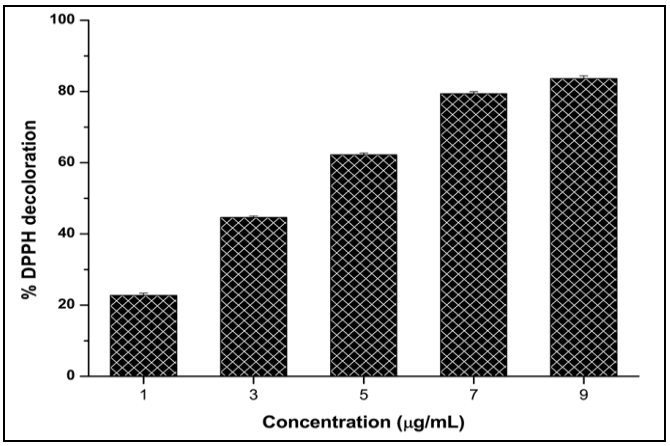

FIG. 3: PERCENTAGE DECOLORATION OF DPPH

Hydroxyl Radical Scavenging Assay:

Hydroxyl radicals are the major active oxygen species causing oxidation of polyunsaturated fatty acid in food and enormous cellular and tissue damage [25]. They are the most reactive of the ROS and are formed from the reaction of various hydroperoxides with transition metal ions. OH radicals induce oxidative damage to proteins, DNA, polyunsaturated fatty acid in membranes and other biological molecules and are known to be capable of abstracting hydrogen atoms from membrane lipids through peroxidation reaction of lipids.

In the present study, curcumin exhibited a concentration dependent scavenging activity against hydroxyl radicals generated (**Fig. 4**). IC_{50} of curcumin in this assay was 30.47µg/mL. This indicates that curcumin invariably could ameliorate oxidative stress associated disorders.

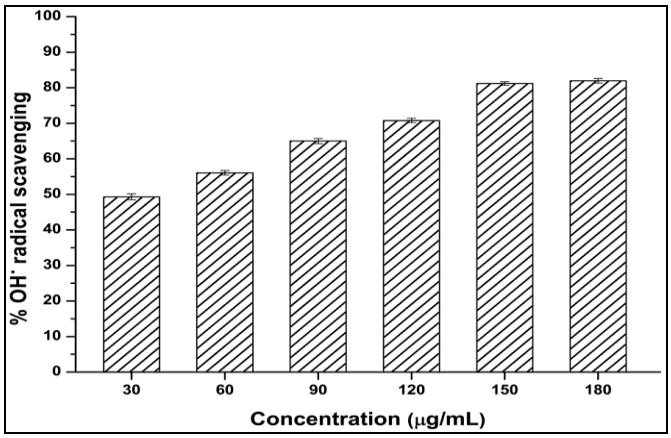

FIG. 4: PERCENTAGE INHIBITION OF OH˚ RADICAL

Nitric Oxide Scavenging Activity Assay:

Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [26]. The absorbance of the reaction mixture decreases as the amount of curcumin increases due to decrease in the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*.

Nitric oxide is a diffusible free radical which plays an important role in the pathogenesis of pain, inflammation, etc. and can act as effect or molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial as well as antitumor activities[27]. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage as seen in inflammatory diseases. Curcumin significantly inhibited nitricoxide in a dose dependent manner (**Fig. 5**) with an IC_{50} of 9.71 µg mL^{-1}.

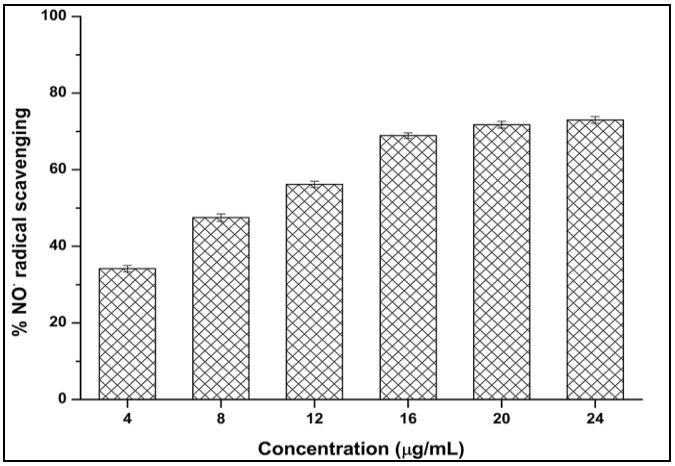

FIG. 5: PERCENTAGE INHIBITION OF NITRIC OXIDE RADICAL

Superoxide Scavenging Capacity:

Superoxide ions are the most common free radicals with increasing concentration under conditions of oxidative stress and are generated either by auto-oxidation processes or by enzymes which produce other cell damaging free radicals and oxidizing agents [28]. The superoxide radicals generated by the hypoxanthine/xanthine oxidase system was determined by monitoring the product of NBT spectrophotometrically. A concentration dependent response of the compound was observed (**Fig. 6**). IC_{50} of was found to be 29.52 μg.mL^{-1}. The result suggested that curcumin effectively scavenged ROS and could protect against oxidative damage.

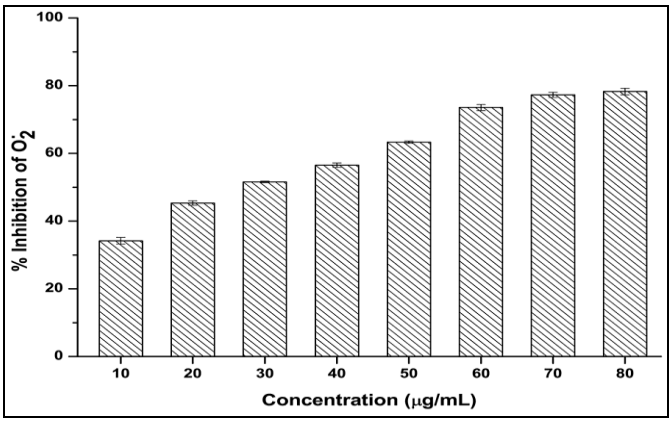

FIG. 6: PERCENTAGE INHIBITION OF SUPEROXIDE RADICAL

This clearly indicates that curcumin is a potential scavenger of excess superoxide radicals produced inside the physiological system which is beyond the balancing capacity of superoxide dismutase.

Earlier workers have been reported that curcumin undergo rapid reactions with thiyl/ sulphur free radicals [29]. Lee *et al*. (2010) have revealed the antioxidant potential of curcumin against radiation-induced oxidative stress [30]. Our investigations involving iron (III) to (II) reducing power assay and OH scavenging are, therefore, additional evidences to conclude that curcumin does indeed possess significant antioxidant potentials.

Radioprotective Properties:

Regarding the radioprotective properties of curcumin, scattered reports exist in the literature suggesting that curcumin confers radioprotection in bacterial cells [31], ameliorates radiation-impaired healing of excisional wounds in mice [32], confers cell cycle-dependent in human cells [33] and affords

protection of radiation-induced cataractogenesis in rats [34]. Curcumin has also been reported to afford protection of mice against γ-ray-induced molecular lesions as evident from reduced apoptotic markers such as DNA fragmentation and caspase-3-activation [35]. Srinivasan *et al*. (2008) have evaluated the radioprotective effect of curcumin analog in primary cultures of isolated rat hepatocytes *in vitro* using single cell gel electrophoresis and showed that curcumin analog pre-treatment could protect hepatocyte cells against gamma radiation. Since curcumin analog in lieu of pure curcumin was used, the results do not fully represent the requisite scientific interest [36].

The ability of curcumin to modulate apoptosis-related cell signalling pathways in cancer cells as a strategy to inhibit tumor progression has been revealed.[37] Evidences of curcumin showing its potential to scavenge various free radical species *in vitro*, reduction of radiation-induced fibrosis thereby increasing survival in mice, and its triggered radiosensitization of cancer cells and radioprotection of normal cells probably places it as a potential candidate against radiological damages [38].

The possibility of using curcumin as a radioprotective agent *via* its ability to reduce oxidative stress and inhibit transcription of genes related to oxidative stress and inflammatory responses has been already raised [39]. Curcumin as a non-genotoxic agent reduced DNA damage, retarded ROS generation and lipid peroxidation, and enhanced the level of antioxidant activity [40]. It is known that cells undergoing apoptosis exhibit specific changes in chromatin condensation, DNA fragmentation, activity of caspase enzymes and nuclear breakdown [41].

Curcumin reduces active caspase-3 and DNA fragmentation induced by gamma radiation by attenuating related signalling pathways [42]. Tawfik *et al*. (2013) have shown that administration of curcumin reduced the effects of γ-ray on DNA fragmentation while caspase-3 cleavage was not affected except the irradiated groups in mice liver cells [35]. Protection of DNA damage in human lymphocytes by curcumin has also been reported [43].

During exposure to ionizing radiation the plasmid DNA suffered strand breaks which converted the super coiled covalently closed circular (CCC) form to open circular (OC) form with a difference in the mobility in the agarose gel. Exposure of pBR322 DNA to gamma radiation at 5 Gy results in the depletion of the CCC form of DNA which was further increased when exposed to 10 Gy (**Fig. 7; Table 1**). Irradiation of the plasmid in the presence of curcumin solution made in 40% ethanol protected from radiation-induced lesions.

The radiation-induced conversion of CCC form to OC form was considerably reduced in the presence of curcumin. Treatment of plasmid pBR322 DNA at different concentrations (10-40 µg/ mL) of curcumin before 1 hour of radiation exposure rendered protection of plasmid DNA in a dose dependent manner by checking the depletion of CCC-form of DNA. Curcumin at a concentration of 10µg/mL was able to provide 62.64% and 57.49% protection of the DNA damages from 5 Gy and 10 Gy radiation exposures respectively. Treatment of DNA with 40µg/mL curcumin was able to provide 79.36% and 74.56% protection from radiation-induced damages when exposed to 5 Gy and 10 Gy respectively. The significant protection of curcumin against radiation-induced pBR322 DNA *in vitro* indicates that it scavenge radiation-induced free radical species in a physico-chemical manner.

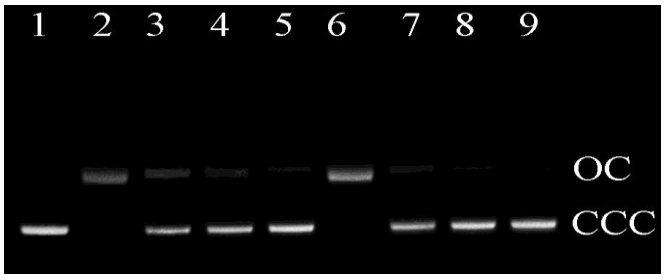

FIG. 7: AGAROSE GEL ELECTROPHORESIS PATTERN OF PBR322 DNA EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION IN THE ABSENCE AND PRESENCE OF CURCUMIN. LANE1: CONTROL; LANE 2: DNA EXPOSED TO 5 GY; LANE 3: DNA WITH 10 *M*G/ML CURCUMIN EXPOSED TO 5 GY; LANE 4: DNA WITH 20 *M*G/ML CURCUMIN EXPOSED TO 5 GY; LANE 5: DNA WITH 40 *M*G/ML CURCUMIN EXPOSED TO 5 GY; LANE 6: DNA EXPOSED TO 10 GY; LANE 7: DNA WITH 10 *M*G/ML CURCUMIN EXPOSED TO 10 GY; LANE 8: DNA WITH 20 *M*G/ML CURCUMIN EXPOSED TO 10 GY; AND LANE 9: DNA WITH 40 *M*G/ML CURCUMIN EXPOSED TO 10 GY. ABBREVIATIONS: 'OC' DENOTES OPEN CIRCULAR OR LINEAR FORM OF DNA AND 'CCC' DENOTES COVALENTLY CLOSED CIRCULAR FORM OR SUPERCOILED FORM OF DNA

The cellular membrane and DNA are the two main targets of radiation-induced lethal effects and mutagenicity. DNA constitutes the primary vital target for cellular inactivation of living systems by ionizing radiation. Damages of cellular DNA by ionizing radiation are mainly strand breaks, elimination of bases and sugar damage. The alkaline comet assay is an elegant and effective technique to monitor the extent of DNA damage and itsprotection. The tail length of the comet is an indicator of DNA damage as smaller molecules move faster on the agarose gel.

Thus, longer tails indicated that the strand breaks were more frequent and DNA was fragmented into several small molecules. Exposure of bone marrow cells to gamma radiation resulted in severe damage as is reflected from the comet scores. Tail DNA and tail length in non-irradiated samples (8.26 ± 0.70 and 1.98 ± 0.13) were increased to 32.50 ± 0.78 and 21.38 ± 0.87 respectively in samples irradiated with 4 Gy (**Table 1**). There was no significant difference observed between the 60% ethanol and non-treated groups.

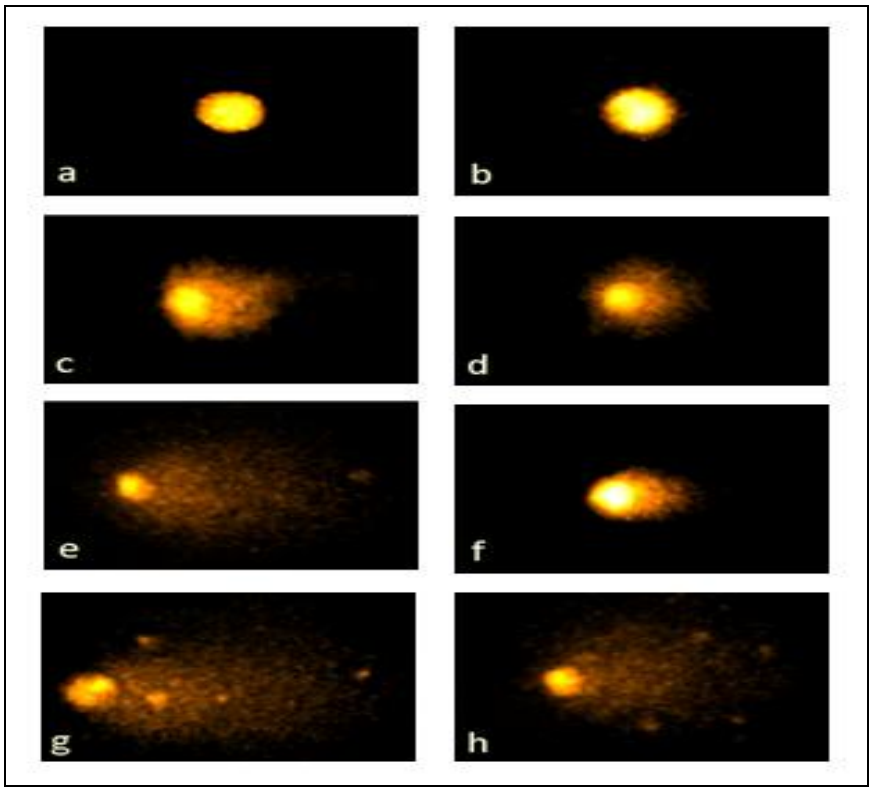

FIG. 8: IMAGES OF COMETS (BONE MARROW CELLS OF RAT) STAINED WITH ETHIDIUM BROMIDE. (A) CELL FROM CONTROL GROUP, (B) 60 % ETHANOL GROUP, (C) 60 % ETHANOL GROUP EXPOSED TO 1 GY, (D) CURCUMIN TREATED GROUP EXPOSED TO 1 GY, (E) 60 % ETHANOL GROUP EXPOSED TO 2 GY, (F) CURCUMIN TREATED GROUP EXPOSED TO 2 GY, (G) 60 % ETHANOL GROUP EXPOSED TO 4 GY, (H) CURCUMIN TREATED GROUP EXPOSED TO 4 GY

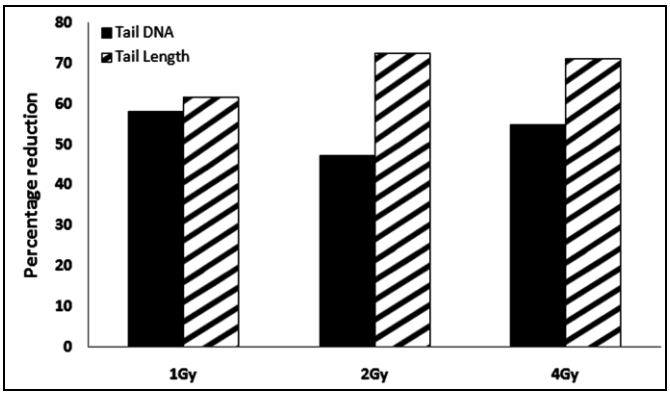

FIG. 9: PERCENTAGE REDUCTION OF RADIATION-INDUCED DNA DAMAGE BY CURCUMIN

Pre-treatment of 60% ethanolic solution of curcumin before irradiation reduced the two comet parameters significantly ($P<0.05$) indicating protection of DNA by curcumin against radiation-induced single-strand breaks. Percentage reductions in radiation-induced DNA damage were found to be 47.26-58.08 % for tail DNA and 61.87-72.64 % for tail length respectively (**Figs. 8** and **9; Table 2**). Thus, the decrease of comet parameters in the presence of curcumin before irradiation indicates its significant role in protection of DNA damages.

TABLE 1: PROTECTION OF PBR322 DNA FROM RADIATION-INDUCED DAMAGES BY CURCUMIN

Treatment	OC form (%)		Protection (%)	
	5 Gy	10 Gy	5 Gy	10 Gy
DNA	83.09	96.46	-	-
DNA + 10 µg/ml curcumin	37.36	42.51	62.64	57.49
DNA + 20 µg/ml curcumin	28.68	34.18	71.32	65.82
DNA + 40 µg/ml curcumin	20.64	25.44	79.36	74.56

TABLE 2: COMET SCORES (TAIL DNA AND TAIL LENGTH) OF RAT BONE MARROW CELLS IN AT DIFFERENT TREATMENTS

Treatment	Tail DNA (Mean ± SE)	Tail length (Mean ± SE)
Control	8.26 ± 0.70	1.98 ± 0.13
60% Ethanol	8.82 ± 0.63	2.23 ± 0.12
Extract	8.43 ± 0.54	1.95 ± 0.11
1Gy	18.66 ± 0.91	9.35 ± 0.61
60% Ethanol + 1Gy	19.32 ± 0.97	9.58 ± 0.72
Extract + 1Gy	12.62 ± 0.85	4.79 ± 0.53
2Gy	22.33 ± 0.95	14.41 ± 0.75
60% Ethanol+ 2Gy	23.20 ± 0.94	15.32 ± 0.68
Extract + 2Gy	15.68 ± 0.84	5.38 ± 0.36
4Gy	32.50 ± 0.78	21.38 ± 0.87
60% Ethanol + 4Gy	34.28 ± 0.81	21.90 ± 0.71
Extract + 4Gy	19.19 ± 0.86	7.58 ± 0.64

Values are expressed as mean ± SE (n=5). Values are statistically significant at $p \leq 0.05$ as determined by Duncan's test. ANOVA does not apply between columns.

Thus, our experiments with gamma radiation administered within the bone marrow syndrome range in rat (1-4 Gy) significantly protected DNA damages by pre-treatment with curcumin in 60% ethanol. Several investigators earlier have interpreted their results at the molecular level involving different biochemical pathways. We believe that curcumin molecules afford protection via molecular pathways as well as physico-chemical processes in which the molecules interact with the radiation-induced reactive species thereby protecting the DNA. Further experiments are under way to resolve the molecular mechanism thereof.

CONCLUSION: Curcumin is a potential therapeutic agent which controls oxidative and non-oxidative damages caused by reactive oxygen and nitrogen species. It shows strong antioxidant activity, reducing power ability and free radical scavenging activity. Protections of DNA *in vitro* and *in vivo* models infer that this natural product has radioprotective property in conformity with its known useful medicinal values. It is a potential candidate molecule which can control post-irradiation damages during radiation countermeasures.

CONFLICT OF INTERESTS: The authors declare that there are no conflicts of interests regarding the publication of this paper.

ACKNOWLEDGEMENT: The authors are thankful to the University Grants Commission, Government of India, New Delhi for providing financial support.

REFERENCES:

1. Manolova Y, Deneva V, Antonov L, Drakalska E, Momekova D and Lambov N: The effect of the water on the curcumin tautomerism: A quantitative approach.

SpectrochimicaActa Part A. Molecular and Biomolecular Spectroscopy 2014; 132: 815-820.
2. Nurfina AN, Reksohadiprodja MS, Timmerman H, Jenie UA, Sugiyanto D and Van der Goot H: Synthesis of some symmetrical curcumin derivatives and their anti-inflammatory activity. European Journal of Medicinal Chemistry 1997; 32: 321-328.
3. Pandey A, Gupta RK and Srivastava R: Curcumin: The Yellow Magic. Asian Journal of Applied Sciences 2011; 4:343-354.
4. Pandey A, Gupta RK, Bhargava A and Agrawal B: Antibacterial Activities of Curcumin Bioconjugates. International Journal of Pharmacology 2011; 7:874-879.
5. Gupta SC, Patchva S, Koh W and Agarwal BB: Discovery of curcumin, a component of the golden spice, and its miraculous biological activities. Clinical and Experimental Pharmacology and Physiology 2011; 39: 283-99.
6. Rajasekaran SA: Therapeutic potential of curcumin in gastrointestinal diseases. World journal of Gastrointestinal Pathophysiology 2011; 2: 1-14.
7. Kalpana C and Menon VP: Curcumin ameliorates oxidative stress during nicotine induced lung toxicity in Wistar rats. Italian Journal of Biochemistry 2004; 53: 82-88.
8. Sai KB, Prema G, Jaideep M, Jayamathi KM, Cherian CN and Ram C. Antioxidant and free radical scavenging activity ofcurcumin determined by using different *in vitro* and *ex vivo* models. Journal of medicinal plant research 2013; 7: 2680-2690.
9. Weber WM, Hunsakar LA, Abcouwer SF, Deck LM and Vander JagtDL. Antioxidant activity of curcumin and related enones. Bioorganic and Medicinal Chemistry 2005; 13: 3811-3820.
10. Negar G, Ali MA and Soheil AE: Nanotechnology-applied curcumin for different diseases therapy. Biomedical Research International 2014;76: 159-161.
11. Sugamura K andKeaney IF: Reactive oxygen species in cardiovascular diseases. Free Radical Biology and Medicine 2011; 51: 978-992.
12. Yue-fei W, Jei W, Jing WU, Ping XU, Yi-qi W, Jun-jie G and Danielle H: *In vitro* antioxidant activity and potential inhibitory action against α-glucosidase of polysaccharides from fruit peel of tea (*Camellia sinensis*L.),Biomedical and Biotechnological2014; 15: 173-180.
13. Halliwell B and Gutteridge JMC: Free radicals in biology and medicine. Oxford University Press, New York, Edition 3, 1999: 246-350.
14. Satish BN and Dilipkumar P: Free radicals, natural antioxidants, and their reaction mechanisms. Royal Society of Chemistry 2015; 5: 27986-28006.
15. Ramalingam S, Devarajan N and Muthugounder SS: Antioxidant Compound Quercetin-3-O-α-L-rhamnoside(1→6)-β-D-glucose (Rutin) isolated from ethyl acetate leaf extracts of *Memecylonedule* Roxb (Melastamataceae). Free Radicals and Antioxidants 2015; 5: 35-42.
16. Oyaizu M: Studies on products of browning reaction. Antioxidant activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition and Dietetics 1986; 44: 307-315.
17. Miliauskas G, Vnskutonis PR and Van Beek TA: Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 2004; 85: 231-237.
18. Smirnoff N and Cumbes QJ: Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 1989; 28: 1057-1060.
19. Garrat DC: The Quantitative Analysis of Drugs. Chapman and Hall, Tokyo, Japan, Edition 3, 1964: 456-458.
20. Lee KW, Kundu JK, Kim SO, Chun K, Lee HJ and Surh Y: Cocoa polyphenols inhibit phorbol ester-induced superoxide anion formation in cultured HL-60 cells and expression of cyclooxygenase-2 and activation of NF-κB and MAPKs in mouse skin *in vivo.* Journal of Nutrition 2006; 136: 1150-1155.
21. Green MR and Sambrook J: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Fourth Edition 2014.
22. Singh NP, McCoy MT, Tice RR and Schneider EL: A simple technique for quantification of low levels of DNA damage in individual cells. Experimental Cell Research 1988; 175: 184-491.
23. Manoharan K and Banerjee MR: β-Carotene reduces sister chromatid exchange induced by chemical carcinogens in mouse mammary cells in organ culture. Cell Biology International Reports 1985; 9: 783-789.
24. Waters MD, Brady AL, Stack HF and Brockman HE: Antimutagenic profiles for some model compounds. Mutation Research1990; 238: 57-85.
25. Marcocci PL, Sckaki A and Albert GM: Antioxidant action of *Ginkgo biloba* extracts EGP761. Methods inEnzymology. 1994; 234: 462- 475.
26. Miller MJ, Sadowska-krowicka H, Chotinaruemol S, Kakkis JL and Clark DA: Amelioration of chronic ileitis by nitric oxide synthase inhibition. TheJournal of Pharmacology and Experimental Therapeutics 1993; 264: 11-16.
27. Liu F and Ng TB: Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Sciences 2000; 66: 725-735.
28. Aurand LW, Boone NH and Giddings GG. Superoxide and singlet oxygen in milk lipid peroxidation. Journal of Dairy Science 1977; 60: 363-369.
29. D'Aquino M, Bullion C, Chopra M, Devi D, Devi S, Dunster C, James G, Komuro E, Kondu S, Niki E, Raza F, Robertson F, Sharma J and Willson R: Sulfhydryl free radical formation enzymatically, by sonolysis, by radiolysis, and thermally: vitamin A, curcumin, muconic acid, and related conjugated olefins as references. Methods in Enzymology 1994; 233: 34-46.
30. Lee JC, Kinniry PA, Arguiri E, Mathew S, Kanterakis S, Chatterjee S, Solomides CC, Javvadi P, Koumenis C, Cengel KA and Christofidou-Solomidou M: Dietary curcumin increases antioxidant defences in lung, ameliorates radiation-induced pulmonary fibrosis, and improves survival in mice. Radiation Research 2010; 173: 590-601.
31. Pal A and Pal AK: Radioprotection of turmeric extracts in bacterial system. ActaBiologicaHungarica 2005; 56: 333-343.
32. Jagetia GC and Rajanikant GK: Effect of curcumin on radiation-impaired healing of excisional wounds in mice. Journal of Wound Care 2004; 13: 107-109.
33. Sebastia N, Montoro A, Hervas D, Pantelias G, Hatzi VI, Soriano JM, Villaescusa JI and Terzoudi GI: Curcumin and trans-resveratrol exert cell cycle-dependent radioprotectiveor radiosensitizing effects as elucidated by the PCC and G2-assay. Mutation Research 2014; 766-767: 49-55.
34. Ozgen SC, Dokmeci D, Akpolat M, Karadag CH, Gunduz O, Erbas H, Benian O, Uzal C and Turan FN: The protective effect of curcumin on ionizing radiation-induced cataractogenesis in rats. Balkan Medical Journal 2012; 29: 358-363.

35. Tawfik SS, Abouelella AM and Shahein YE: Curcumin protection activities against γ-rays-induced molecular and biochemical lesions. BMC Research Notes 2013; 6: 375-384.
36. Srinivasan M, Sudheer AR, Rajasekaran KN and Menon VP: Effect of curcumin analog on gamma radiation-induced cellular changes in primary culture of isolated rat hepatocytes *in vitro*. Chemical Biological Interactions 2008; 176: 1-8.
37. Chen J, Wang FL and Chen WD:Modulation of apoptosis-related cell signalling pathways by curcumin as a strategy to inhibit tumor progression. Molecular Biology Reports 2014; 41: 4583-8594.
38. Kma L: Plant extracts and plant-derived compounds: promising players in a countermeasure strategy against radiological exposure. Asia Pacific Journal of Cancer Prevention 2014; 15: 2405-2425.
39. Jagetia GC: Radioprotection and radiosensitization by curcumin. Advances in Experimental Medicine and Biology 2007; 595: 301-320.
40. Biswas J, Sinha D, Mukherjee S, Roy S, Siddiqi M and Roy M: Curcumin protects DNA damage in a chronically arsenic-exposed population of West Bengal. Human Experimental Toxicology 2012; 29: 513-524.
41. Tang HL, Tang HM, Mak KH, Hu S, Wang SS, Wong KM, Wong CS, Wu HY, Law HS, Liu K, Jr. Talbot CC, Lau WK, Montell DJ and Fung MC: Cell survival, DNA damage, and oncogenic transformation after a transient and reversible apoptotic response. Molecular Biology of the Cell 2012; 23: 2240-2252.
42. Zhang R, Kang KA, Kang SS, Park JW and Hyun JW: Morin (2,3,4,5,7-pentahydroxyflavone) protected cells against gamma radiation-induced oxidative stress. Basic Clinical Pharmacology and Toxicology 2011; 108: 63-72.
43. Nayereh S, Monireh H and Seyed JH:Protective effects of curcumin against genotoxicity induced by 131-iodine in human cultured lymphocyte cells. Pharmacognosy Magazine 2014; 10: 106-110.

How to cite this article:
Singh PP, Devi KR, Devi MM, Thokchom DS and Sharma GJ: Protection of Low Let Radiation-Induced DNA Damage in Rat Bone Marrow Cells by Free Radical Scavenger Curcumin. Int J Pharm Sci Res 2016; 7(3): 1168-78.doi: 10.13040/IJPSR.0975-8232.7(3).1168-78.