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Original Article

STUDIES ON BIOAVAILABILITY ENHANCEMENT OF CURCUMIN

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ABSTRACT

Objective: The objective of the present work was to improve aqueous solubility and *in vivo* bioavailability of curcumin and structural analogues of curcumin such as potassium, calcium, magnesium salts and nitro derivative.

Methods: Structural analogues of curcumin were prepared by reaction of curcumin with potassium chloride, magnesium chloride hexahydrate and calcium chloride dihydrate in a suitable solvent. The nitro derivative synthesized by treating curcumin with sulphuric acid and nitric acid. The prepared analogues were evaluated for melting behavior, solubility, UV spectrophotometry, partition coefficient, moisture content, cellular uptake, FTIR analysis, antimicrobial activity and *in vivo* bioavailability in the rat.

Results: Chemical modification of curcumin increased the saturation solubility to 11.6, 16.5, 21.5, 28.0 µg/ml in calcium salt, magnesium salt, potassium salt and nitro derivative respectively, against 8.6 µg/ml of curcumin. The analogues were chemically stable as curcumin analyzed by FTIR spectrophotometry. Increased cellular uptake, as well as enhanced antimicrobial activity, was demonstrated by modified curcumin analogues. Moreover, significant improvement in plasma levels was estimated with nitro derivative.

Conclusion: The present work recommends that nitration of curcumin improves aqueous solubility which may improve absorption and *in vivo* bioavailability.

Keywords: Curcumin, Structural analogues, Bioavailability, Antimicrobial activity

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INTRODUCTION

Curcumin, a naturally occurring polyphenolic diferuloylmethane extracted from the rhizomes of plant *Curcuma longa* Linn. (Family: Zingiberaceae) has potential in the prevention and therapeutic interventions of several pathological conditions including respiratory diseases, inflammation, liver disorders and diabetic wounds. It prevents a variety of carcinogen-induced cancers in rodents by suppressing the mutagenic effects of carcinogens [1]. In addition, the hepato-and nephro-protective, thrombosis suppressing, myocardial infarction protective, hypoglycemic, and antirheumatic effects of curcumin are also reported [2-4]. Several animal and human studies have demonstrated the safety for curcumin at very high doses (12 g/d) [5-7] which makes it a potential compound for treatment and prevention of a wide variety of human diseases. In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent due to its low bioavailability presenting a significant pharmacological obstacle for clinical application. It is a hydrophobic compound with poor aqueous solubility and low absorption. It undergoes rapid metabolism and rapid systemic elimination further diminishing the bioavailability. The solubility of curcumin in water especially at acidic and physiological pH is extremely low (11 ng/ml). It undergoes rapidly hydrolysis under alkaline conditions and readily decomposed when exposed to bright light, high temperature or oxidative conditions [8]. Based on its poor aqueous solubility and permeability, curcumin can be classified as a BCS class IV molecule [9]. Effective pharmacokinetics is a surrogate for pharmacodynamics and is a determinant of therapeutic outcome. Although curcumin showed potential pharmacodynamics, it has poor pharmacokinetic characteristics. Many attempts have been made to increase solubility, absorption (permeability) and stability of curcumin in order to increase bioavailability.

Although many clinical trials for curcumin are currently ongoing [10], its clinical advancement of is hampered by its poor water solubility and short biological half-life and low bioavailability in both plasma and tissues [11-13]. The oral bioavailability of curcumin and synthetic structural analogues of curcumin is very low (only 1% in rats) [14-16].

The motivation for building curcumin analogues is based upon its rapid metabolism and conjugation in the liver and its excretion through feces which limit the systemic bioavailability. In order to overcome these limitations, several approaches have been attempted including the combination of curcumin with adjuvants such as piperine [17] and the development of delivery vehicles consisting of liposomes nanoparticles a phospholipid complex [18-20]. Several efforts for improving the systemic and tissue bioavailability of curcumin have largely been unsuccessful. The aim of the present work is to improve aqueous solubility and *in vivo* bioavailability of curcumin by using its structural analogues.

MATERIALS AND METHODS

Curcumin was received as gift sample from the Baidhyanath Pvt. Limited (Nagpur, India). Potassium hydroxide, calcium chloride dehydrate, magnesium chloride hexahydrate, and nitric acid, sulphuric acid were obtained from Merck Specialities Private Limited, Fisher Scientific and HiMedia Laboratories (Mumbai, India).

Preparation of structural analogues of curcumin

The structural analogues of curcumin were prepared by reaction of curcumin with potassium chloride, magnesium chloride hexahydrate and calcium chloride dihydrate in a suitable solvent (methanol, water). Salts were recrystallized and the resulting crystals were washed and dried [21]. The nitro derivative of curcumin synthesized, washed and dried [22].

Preparation of potassium salt

Aqueous 80 ml KOH was added to a solution of 5 g curcumin in 40 ml chloroform. The mixture was sonicated for 4 h. The organic layer was evaporated and aqueous layer was washed with a respective solvent. Salt was recrystallized with ethanol-water mixture and dried [23].

Preparation of calcium salt

A solution of calcium chloride dihydrate in 50 ml of water was added to a solution of 20 g potassium salt in 250 ml warm water. Stirring

was continued for 3 h, and then the crystals were filtered and washed with water. By using Potassium salt, calcium salts was prepared [23].

Preparation of magnesium salt

A solution of magnesium chloride hexahydrate in a 100 ml of water was added with stirring to a solution of 20 g potassium salt in 250 ml warm water. Stirring was continued for 3 h, and then the crystals were filtered and washed with water [23].

Preparation of nitro derivative

Curcumin was reacted with the mixture of sulphuric acid and nitric acid for 6 h in the reaction vessel. Then the mixture was filtered, washed and dried [23].

Evaluation of structural analogues of curcumin

Melting point determination

Melting point of curcumin and its structural analogues were determined in an electric melting point apparatus (Elico, India) by capillary method.

UV spectrophotometric analysis

Analysis of curcumin and its structural analogue in methanol was carried out using UV Spectrophotometer (Jasco, U. S. A.).

Solubility study using the shake-flask method

Saturated solutions of curcumin and its structural analogues were prepared by mixing an excess of solid solute in 10 ml distilled water. Then these mixtures were agitated in mechanical shaker thermostated at 30 °C±0.1 ° C for 24 h. The filtered samples were diluted and analyzed by UV Spectrophotometry [24].

Moisture content determination

Approximately 1g of the sample was dehydrated in hot air oven at 60 °C and reweighed at an interval of 10 min till the constant weight for three consecutive determinations and % moisture loss was calculated.

Partition coefficient determination

The partition coefficient was determined using the shake-flask method**.** An excess amount of curcumin was shaken in an equal volume of octanol and water in a separating funnel. Then it was kept aside to separate the layers octanol and water. The separated layers were analyzed using uv-spectrophotometer at 271 nm wavelength.

Cellular uptake determination

The cellular uptake of curcumin and its salts/derivative were examined by UV spectrophotometry. A constant concentration (100 µl) of curcumin and its salts were made and mix with an equal amount of stock solution of RBC (10 ml in 100 ml). The mixture was centrifuged at a fixed rpm at a 37 °C for a 10 min. Supernatant was collected and observed against the blank and saline solution [24]

FTIR analysis

Curcumin was dried in a hot air oven at 50 °C for 1 h. The samples were prepared by mixing it thoroughly with potassium bromide. This physical mixture was compressed under of 10 ton/nm^2 and converted in a circular disc. This disc was then placed in a scanning slot of Fourier Transform Infra-red (FTIR) spectrophotometer (Shimadzu 1800) and scanned at range from 400 to 4000 cm^{-1}. The spectrum was then compared with the spectrum of the reference standard.

Antimicrobial assay

Staphylococcus aureus and *Escherichia coli* (HiMedia Laboratories Mumbai, India). were used as the test organisms. Antimicrobial efficiency was determined by the Cup-Plate method. Sterile solutions of curcumin and its analogues were diluted at different concentration these solutions were poured into cups bored into sterile nutrient agar previously seeded with the test organisms, after allowing diffusion of the solution for 2 h, the agar plates were incubated at 37 °C for 24 h. The zone of inhibition (ZOI) measured around each cup and was compared with that of control.

***In vivo* bioavailability assessment**

Male Sprague rats weighing 250±20 g were randomly divided into 5 groups and fasted for 10-12 h prior to experiments, although they were allowed free access to water. All the experimental protocol was carried out according to the guidelines of CPCSEA (853/AC/04/CPCSE). A feeding tube was inserted into the mouth of rat. The animals in the first groups were orally administered 1 ml of 0.5 carboxymethyl cellulose (CMC) saline, an aqueous suspension containing curcumin at a dose of 100 mg/kg, and those were remaining in the other groups were received potassium salt, calcium, magnesium, and nitro derivative respectively. Then 50 µl of blood was collected from tail vein at a pre-determined interval for a 8h and centrifuged at 12000 rpm for 10 min.

Plasma was mixed with ethyl acetate and extracted twice with it by vigorous shaking on a mechanical shaker for 10 min. The upper organic layer was removed and placed in a cleaned centrifuge tube. The combined organic phase was then evaporated at room temperature and the residue was reconstituted with 100 µl mobile phase. The 50 µl of supernatant was analyzed by HPLC (Analytical). In HPLC mobile phase, THF (40 %) and water containing 1.2 % citric acid were used. The flow rate of 1 ml/min was used [24].

RESULTS AND DISCUSSION

Melting points for curcumin and its potassium, magnesium, calcium salts and nitro derivative were found to be in the range of 182-184 °C, 320-325 °C, 310-312 °C, 330-332 °C and 210-220 °C respectively. Increased melting point of the salts might be a result of ionic nature of the salts. In addition, increased molecular weights of nitro derivative also resulted in increased melting temperature and hence require more energy for breaking of ionic bonds or ion-dipole or Vander wall interaction. The distinguishing and higher melting points confirmed the successful synthesis of curcumin derivative and salt.

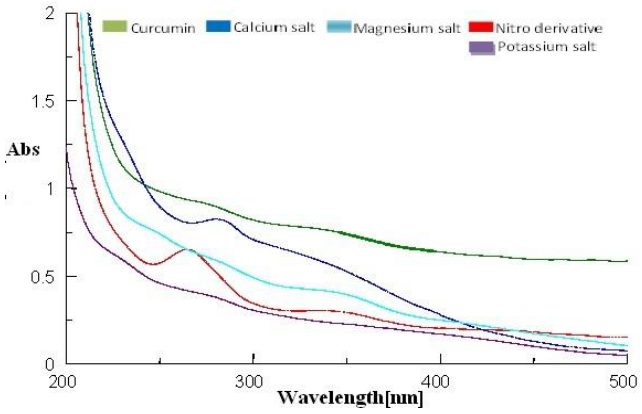

Fig. 1: Overlain of UV spectrum of curcumin and its potassium, calcium, magnesium salts and nitro derivative

The UV spectrum was scanned for Curcumin at λ_{max} 271 nm. As shown in fig. 1, UV spectra of curcumin and its analogues (salt and derivatives) exactly overlap to each other suggesting the intactness of the basic moiety of curcumin structure in its analogues. Moreover, the spectrum pattern in nitro derivative and calcium salt of curcumin showed sharp peak than other analogues. This was due to the group present in the nitro derivative of curcumin which shows the electronic transitions and responsible for giving sharp peak.

The solubility for curcumin and its calcium, magnesium, potassium salts, and nitro derivative were found to be 8.690±0.068, 11.655±0.22, 16.502±0.363, and 21.551±0.162, 28.050±0.202 µg/ml in water respectively. The solubility of calcium, magnesium, potassium salts and nitro derivative of curcumin observed increases in comparison with the curcumin. The enhanced solubility can be correlated with salt formation. Thus salt particles can get easily ionized, dispersed and immediately dissolved in presence of water. The maximum solubility was observed in nitro derivative compared with curcumin and its salts.

The %moisture content for curcumin and its potassium, calcium, magnesium salts and nitro derivative were found to be 0.10, 0.22, 0.44, 0.51 and 0.14 %. The hygroscopic nature of curcumin analogues observed as increased compared with curcumin and maximum increment was found in magnesium salt of curcumin.

The partition coefficient (octanol/water) of curcumin, potassium salt, calcium salt, magnesium salt, and nitro derivative of curcumin were found to be 0.92085±0.121, 0.46924±0.132, 0.628095±0.121, 0.84343±0.201 and 0.29229±0.111, respectively. From the above observations, with the decreasing partition coefficient the being lowest with nitro derivative. The partitioning of salt and derivative of curcumin were founds towards the higher level in the water phase because of its greater water solubility. Hence, the coefficients of salts and derivatives observed at lower range than that of curcumin. As higher water solubility required for the absorption of the drug through the gastrointestinal tract was shown by prepared curcumin analogues compared with curcumin.

The cellular uptake of curcumin, and its potassium, calcium, magnesium salts, and nitro derivative were found to be 86.86±0.755, 99.05±0.055, 98.13±0.107, 97.32±0.154 and 92.56±0.427 % respectively. The highest cellular uptake was observed in potassium salt, 99.05±0.055 % of curcumin was found to be taken up by RBCs from the solution [24].

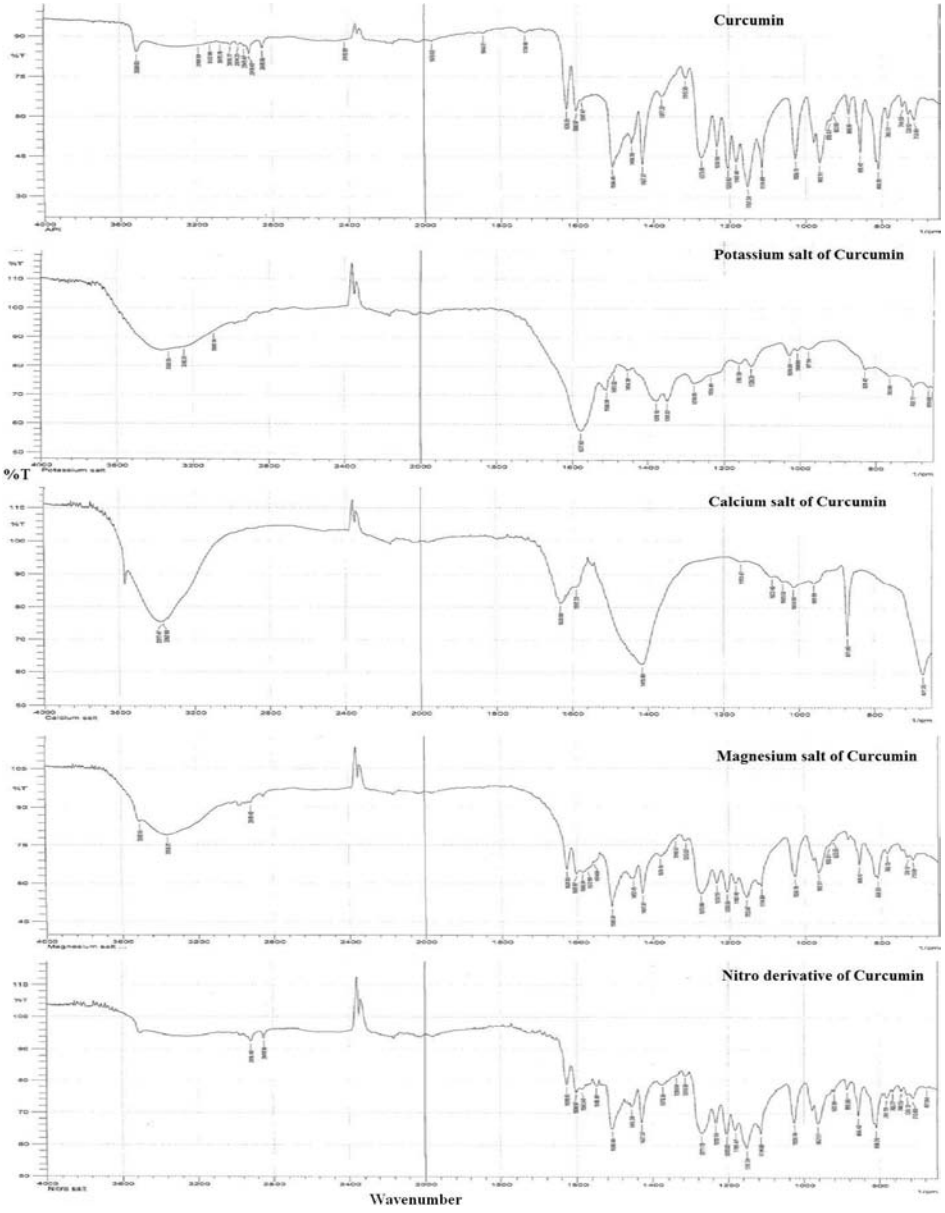

Fig. 2: FTIR spectrum of curcumin and its potassium, calcium, magnesium salts and nitro derivative

Table 1: FTIR peaks of curcumin and its potassium, calcium, magnesium salts and nitro derivative

Functional groups	Wavenumber (cm^{-1})				
	Curcumin	Potassium salt of curcumin	Calcium salt of curcumin	Magnesium salt of curcumin	Nitro derivative of curcumin
O-H stretching	3508	3329	3377	3502	3502
C=O, C=C	1626	1577	1629	1626	1628
Aromatic C=O	1601	1508	1591	1600	1600
Phenol, C-O	1429	1454	1415	1427	1427
Enol	1272	1278	-	1273	1271
NO	-	-	-	-	1548

As shown in fig. 2 and table 1, the band at 3508 cm^{-1} indicates the presence of the hydroxyl group in the curcumin. The band of (phenol) alkanes (C-H) observed at 1350-1512 cm^{-1}. FTIR spectrum of curcumin exhibited the absorption peaks 3508, 1626, 1601 and 1429, 1272 cm^{-1}assigned to–OH, C=O, phenol, enol, and C -O-C stretching respectively. The results were found to be concurrent with a reference spectrum of curcumin [25].

FTIR spectrum of calcium salt showed the absorption peaks 3377, 1626, 1591 and 1415 cm^{-1}associated with O-H, C=O, aromatic C=O, phenol, enol stretching respectively. The O-H group shifting was observed in calcium salt from 3508 to 3377 cm^{-1}, C=O group was shifted towards the lower wavenumber side from 1629 to 1626 cm^{-1}, C-O shifted from 1429 to 1415 cm^{-1}, enolic functional group is missing in calcium salt may be due to ionic nature and replacement of hydrogen ion by calcium cation. Moreover, retention of O-H and C=O groups indicated the presence of curcumin in its calcium salt in a stable form.

FTIR spectrum of potassium salt of curcumin illustrated the absorption bands at 3329, 1577, 1508 and 1454, 1278 cm^{-1}allocated to –OH, C=O, aromatic=O, phenol, enol stretching respectively. The O-H group shifting was observed in potassium salt from 3508 to 3329 cm^{-1}, C=O group was shifted towards the lower wavenumber from 1629 to 1577 cm^{-1}, C-O shifted from 1429 to 1454 cm^{-1}, enolic group moved towards higher wavenumber from 1272 to 1278 cm^{-1}. In these, all the functional groups of curcumin retained by its potassium salt suggested its chemical stability.

FTIR spectrum of the magnesium salt of curcumin showed the absorption peaks 3508, 1626, 1600 and 1427, 1273 cm^{-1}assigned to –OH, C=O, aromatic=O, phenol, enol stretching respectively. The O-H group shifting was observed in magnesium salt from 3508 to 3502 cm^{-1}, C-O altered from 1429 to 1427 cm^{-1}. In these, all the functional groups of curcumin retained by its magnesium salt suggested its chemical stability.

FTIR spectrum of nitro derivative exhibited the absorption bands 1628, 1626, and 1427, 1271 cm^{-1}assigned to –OH, C=O, aromatic =O, phenol, enol stretching respectively. The C-O shifted from 1429 to 1427 cm^{-1}, enolic group shifts towards lower side from 1272 to 1271 in nitro derivative. The functional groups of curcumin retained by its nitro derivative suggested its chemical stability. Additionally, the peak at 1548 cm^{-1} observed assisted with N-O, suggested the formed derivative of curcumin was observed as chemically stable.

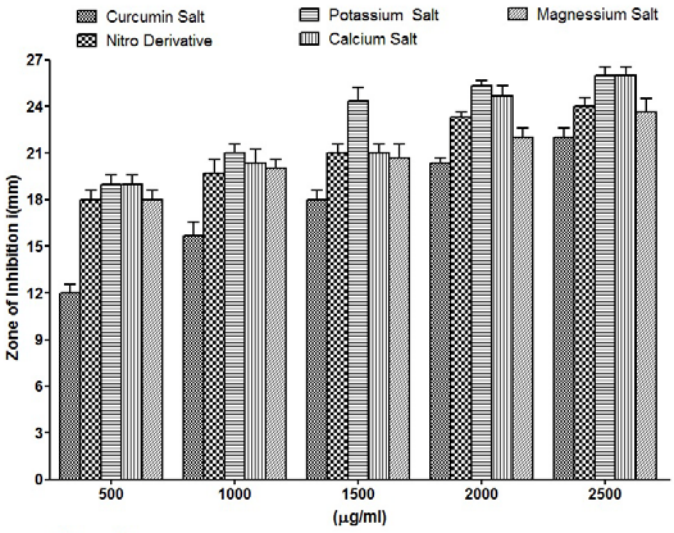

Fig. 3: Antibacterial activity of curcumin and its potassium, calcium, magnesium salts and nitro derivative, *values are expressed as mean+SEM

Table 2: Pharmacokinetic parameters calculated from the plasma curcumin profile following single oral administration of curcumin, its salts and derivative

Compounds	C max ng/ml	T max (min)	t½ (min)	AUC 0-t ng/mlmin
Curcumin	148.41	70	147.35	23673.6
Potassium salt of Curcumin	84.91	60	56.87	52294.6
Calcium salt of Curcumin	97.73	70	163.74	13822.1
Magnesium salt of Curcumin	112.20	70	114.925	116805
Nitro derivative of Curcumin	240.17	60	125.12	35971

*Values are expressed as mean+SEM and n=12

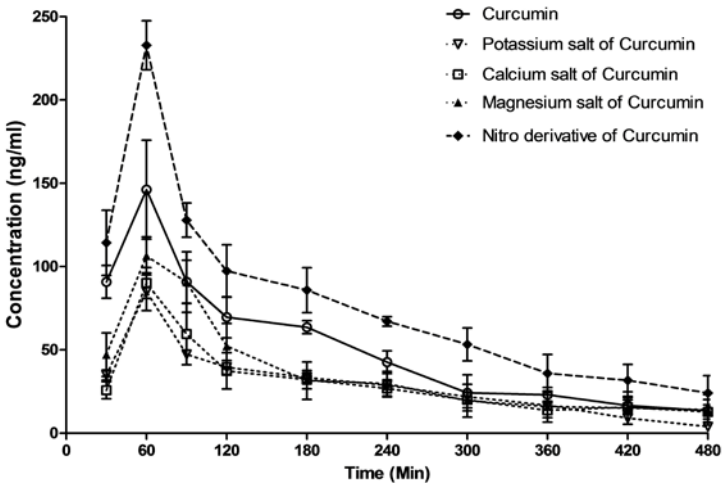

Fig. 4: Plasma concentration time profile of curcumin and its potassium, calcium, magnesium salts and nitro derivative

Curcumin analogues showed greater zones of inhibition than curcumin observed in fig. 3. Moreover, the *in vitro* antibacterial efficacy of the curcumin was not affected by the salt and derivative formation, suggesting the intactness of the necessary structural characteristics required for antibacterial activity [25].

As shown in fig. 4 and table 2, the Cmax of curcumin was found to be 148.41 ng/ml. Cmax was increased from 148.41 ng/ml to 240.17 ng/ml in case of the nitro derivative. However, in the case of *AUC*, it was increased from 23673.6 to 35971 ng/mlmin. There was no significant difference in the time taken to reach the peak concentration (*T*max) of curcumin and their analogues. No significant result was obtained in case of potassium, magnesium and calcium salt. One of the main reasons for the enhance curcumin oral bioavailability by curcumin analogues is the excellent efficiency of curcumin analogues in improving the drug solubility and in increasing the dissolution rate [26-28].

CONCLUSION

Curcumin possesses diverse pharmacological activities. Its utility limits due to its poor water solubility, poor bioavailability and rapid metabolism. In consideration to improve aqueous solubility and *in vivo* bioavailability, structural analogues of curcumin such as potassium, calcium, magnesium salts and nitro derivative were prepared and evaluated. In comparison with curcumin, its analogues exhibited more aqueous solubility. The enhanced solubility data were supported by UV spectral analysis, partition coefficient determination as well as improved cellular uptake by RBC. The analogues were chemically stable as curcumin, which were analyzed by FTIR. The curcumin analogues showed greater antibacterial activity compared with curcumin. The *in vivo* bioavailability assessment showed that nitro derivative of curcumin illustrated greater C max and AUC than curcumin, suggests improved bioavailability and limits metabolism. The present work recommends that nitration of curcumin improves aqueous solubility which may improve absorption and *in vivo* bioavailability in rat.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

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