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Gastroprotective Effect of Nanocurcumin Particles against Indomethacin-induced Gastric Ulcer in Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Author NTHT designed the study, conducted the experiments, performed the statistical analysis and wrote the first draft of the manuscript. Author HLS conceptualized, managed the literature searches, supervised the study and revised the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

This study aims to investigate the gastroprotective properties of nanocurcumin against indomethacin-induced gastric ulcer in mice. Nanocurcumin particles were prepared by top down method, yielding up to 15% (w/w), and the size was defined less than 100 nm in diameter. The particles were characterized by using scanning electron microscope (SEM), transmission electron microscope (TEM), dynamic laser light scattering (DLS), and X-ray diffraction (XRD). Significant gastroprotective properties of prepared nanocurcumin against indomethacin-induced stomach ulcer in mice (20 mg/kg, body weight) was observed by evaluating the levels of LPO, GSH, CAL and total protein in stomach and histological examining stomach of mice. It is believed that nanocurcumin particles are capable of healing mucosal injury inhibiting acid secretion, and reducing free radicals against indomethacin-induced injury stomach in mice.

Keywords: Nanocurcumin; curcumin; indomethacin; gastric ulcer; mice model.

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1. INTRODUCTION

Gastric ulcer is a major health hazard in terms of both morbidity and mortality. Gastric ulcers are benign lesions on the mucosal epithelium upon exposure of stomach to excess acid and aggressive pepsin activity. Untreated gastric ulcer is capable of inducing upper gastrointestinal bleeding. Common causes include the bacteria *Helicobacter pylori* (*H. pylori*) and non-steroidal anti-inflammatory drugs (NSAIDs). Other less common causes could be smoking, alcohol and stress due to serious illness [1]. Turmeric is widely used in traditional oriental medicine, especially in India, China and also in Thailand. It has been traditionally used to treat many diseases including gastric ulcer, anorexia, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis; and externally in the prevention and treatment of skin diseases [2]. Curcumin, a natural bioactive polyphenol compounds, was extracted from the rhizome of turmeric (*Curcuma longa* Linn.) (*C. longa*) that belongs to family Zingiberaceae, a plant grown in tropical Southeast Asia. Curcumin has been defined as the most active component in *C. longa* and has considerable gastroprotective and antiulcerogenic effect. The antiulcer activity of curcumin was displayed by attenuating the different ulcerative effectors including gastric acid hypersecretion, total peroxides, myeloperoxiase activity, IL-6, and apoptotic incidence, along with its inhibitory activity for pepsin [3]. Yadav et al. reported that curcumin can block indomethacin, ethanol, and stress-induced gastric ulcer and can prevent pylorus-ligation-induced acid secretion [4].

Curcumin has been shown to have a wide spectrum of pharmacological effects including anti-oxidant [5], anti-inflammatory, antimicrobial and anticancer properties [6]. Curcumin has been used as a dietary supplement as well as a therapeutic agent in Chinese medicine and other Asian medicines for centuries. For curcumin to exhibit its therapeutic effects in the human body, a person is required to swallow between 12 and 20 g of curcumin everyday [7]. It is well known that curcumin is low aqueous soluble, which is about 0.021 mg/mL [8]. Major reasons contributing to the low plasma and tissue levels of curcumin appear to be due to poor absorption, intestinal metabolism, hepatic metabolism and rapid systemic clearance [9].

Nanotechnology is the most effective tool to enhance the water solubility of curcumin, thereby improving dispersibility, absorption and bioavailability of curcumin. Nanoparticles are easier to pass through cell membranes in organisms and get interacted rapidly with biological systems [10]. Thus, the aim of this study was to prepare nanocurcumin particles by top down method and evaluate the gastroprotective effect of nanocurcumin on indomethacin-induced gastric ulcer in mice.

2. MATERIALS AND METHODS

2.1 Materials

Curcumin (purity > 95%) was procured from Friendship Joint-stock Company (Hoan Kiem, Ha Noi, Vietnam). Cremophor RH40 (RH40) was purchased from Sigma-Aldrich Company. Polyvinyl pyrrolidone (PVP) K-30, poly vinyl alcohol (PVA, Mw = 85000 g/mol), polyethylene glycol 6000 (PEG), polysorbate 80 (Tween 80), polysorbate 20 (Tween 20) and sodium lauryl sulphate (SLS) were obtained from HiMedia, Ltd., Mumbai, India.

2.2 Induction of Gastric Ulcer and Treatment on Mice Model

Healthy Swiss mice *Mus musculus var*. *albino* weighing 18 – 22 g were procured from Pasteur Institute of Ho Chi Minh City. They were housed in clean cages and had free access to standard pallet diet and water *ad libitum.* During the experiment, mice were kept in a controlled environmental condition with 12 h of light and dark cycle. All mice were divided into four groups of ten mice each. Gastric ulceration in mice of groups L1 – L3 was induced by oral administration of indomethacin (10 mg/kg body weight) dissolved in Tris buffer [11]. Group L4 which served as the normal control received only the vehicle oral dose of Tris buffer. Mice in the group L1 received nanocurcumin (20 mg/kg body weight), while in group L2, mice received curcumin (20 mg/kg body weight) once daily by oral administration 12 hours after starting indomethacin treatment. Mice in group L3 (ulcerated control) were given indomethacin only. Twenty four hours after the last dose of the test samples, stomach sample removed from each mouse was fixed in 10% buffered formalin for more than 24 h. The samples were then stained with hematoxylin and eosin (H&E) for histological evaluation at the Department of Histology – Embryology of University of Medicine and Pharmacy – Ho Chi Minh City. The pathological changes in the gastric tissues were observed

under a light microscope. All animals were maintained in accordance with the Animal Experimental Hand Book at Cellular Reprogramming Laboratory, International University, Vietnam National University of Ho Chi Minh City (http://crl.bio.hcmiu.edu.vn/about-us/facilities/) and additionally with the Guide for the Care and Use of Laboratory Animals (8th edition) [12].

2.3 Methods

2.3.1 Preparation of nanocurcumin particles

The nanocurcumin particles were prepared by top down method. 3 g of absolute Tween 80 was added to 250-mL Duran containing 15 g of curcumin powder, 82 g of distilled water and 200 g of glass beads (1 mm in diameter). The mixture was dispersed by Rock Tumbler Unitized Jar Mill machine (U.S. STONEWARE 755RMV, U.S) for 120 hours at 300 rpm, room temperature [13,14]. The mixture was then passed through a stainless steel strainer to remove glass beads. The prepared nanocurcumin particles were characterized using SEM, TEM, DLS and XRAY techniques.

2.3.2 Determination of lipid peroxidation (LPO)

The level of LPO in stomach was measured in terms of thiobarbituric acid reactive substances by the method of Okhawa et al. [15]. The content of LPO was expressed in n mol/mg that was recorded at 532 nm.

2.3.3 Determination of reduced glutathione (GSH) content

The level of reduced GSH was estimated by the method of Moron et al. [16]. The absorbance was recorded at 412 nm with levels expressed as nmol/mg of protein.

2.3.4 Determination of catalase (CAT) activity

Catalase activity was assayed in the stomach by the method of Aebi et al. [17]. The content was estimated at 240 nm by monitoring the disappearance of H_2O_2.

2.3.5 Estimation of protein

Total protein contents in stomach were measured by the method of Lowry et al. [18]. The absorbance was recorded at 680 nm.

2.4 Statistical Analysis

Analysis of variance (ANOVA) was used in analyzing the data generated by this study. All analysis was made with the statistical software Statgraphics centurion XV. Results were expressed as means ± standard deviation. Values of $p<0.05$ were regarded as being significant.

3. RESULTS AND DISCUSSION

The nanocurcumin particles were prepared with proper ingredients and the particles sizes were analyzed and the distribution of nanoparticles was examined by the techniques of scanning electron microscope (SEM), transmission electron microscope (TEM), dynamic laser light scattering (DLS), and X-ray diffraction (XRD). TEM was used to inspect nanocurcumin particles that showed the smaller particle size around 50 nm could be observed (Fig. 2B). Meanwhile, as can be seen from the SEM image (Fig. 2A), the nanocurcumin particles are found to be smooth and shape of spherical to oval. DLS of an aqueous dispersion of nanocurcumin revealed the formation of nanoparticles with an average diameter of 90.5 ± 36.9 nm (Fig. 1B). As a result, the prepared nanocurcumin particles could be well dispersed in water. The products have been stored at room temperature over 10 months without any decomposition or aggregation.

Fig. 3 presents the XRD graphs of curcumin and nanocurcumin particles indicating that the nanocurcumin was successfully produced. The result of X-Ray Diffraction (XRD) D8 ADVANCE type (BRUKER-AXS Germany, 2007) shows nanocurcumin particles were successfully prepared as both standard peaks of curcumin and nanocurcumin have the same the retention time. Data from this XRD analysis also show that curcumin possesses peaks in the 2θ range of 5 – 40° implying its crystal nature (Fig. 3A), and meanwhile the same results have been observed in the nanocurcumin particles (d = 11.20; 10.05; 7.27; 6.09; 5.16; 4.20; 3.82; 3.61. These findings strongly confirm that both nanocurcumin and curcumin had the identical chemical structure.

Indomethacin is considered a risk factor for developing gastric ulcers. Indomethacin also triggers imbalances in cellular antioxidant process. Indomethacin is known to produce necrotic lesions in the gastric mucosa of animals by a direct toxic effect thereby reducing the

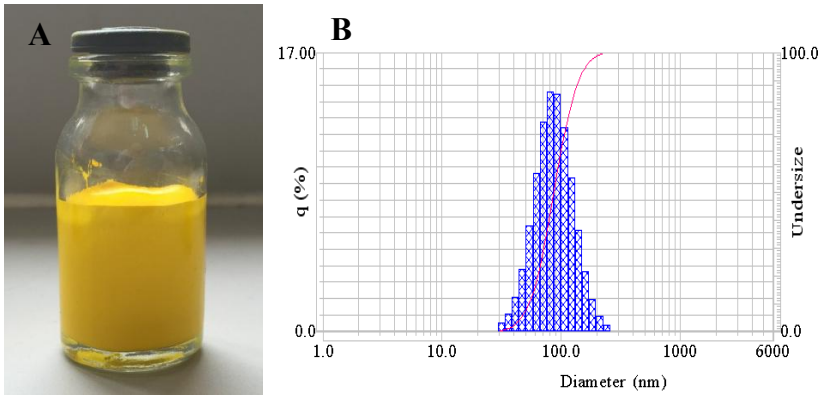

Fig. 1. (A) Nanocurcumin solution 15% and (B) Dynamic laser light scattering (DLS) results of nanocurcumin solution

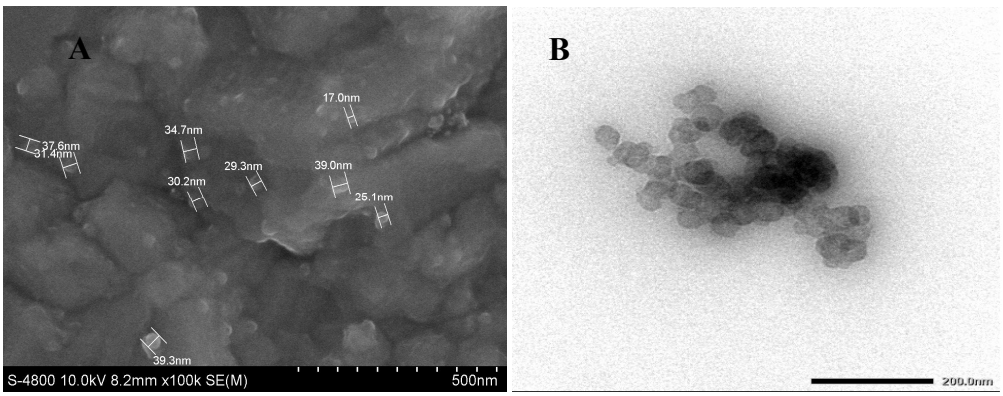

Fig. 2. (A) Nanoparticles size characterization using Field emission scanning electron microscopy (FE-SEM) and (B) Transmission electron microscopy (TEM) of the nanoparticles confirms a narrow size distribution

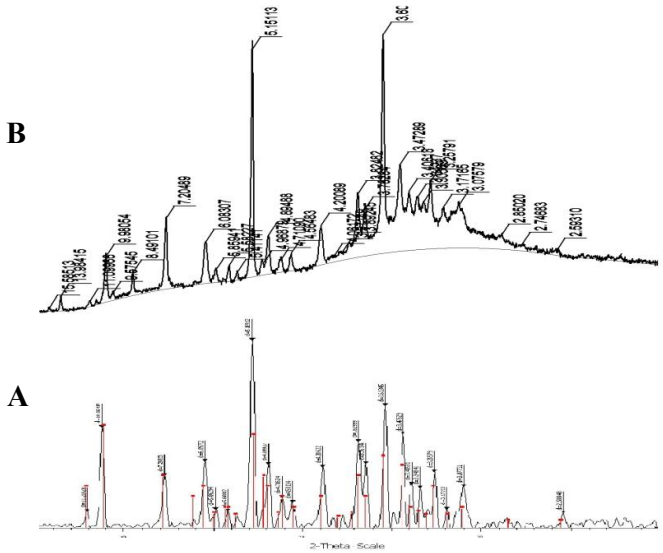

Fig. 3. XRD graph showing the crystalline state of curcumin (A) and nanocurcumin (B)

secretion of bicarbonates and depleting gastric mucus production in animals. The gastric tissue morphologies of mice of group L1 –L4 were also observed macroscopically (Fig. 4A – D). The gastric tissue morphologies of mice from control group presented normal color (Fig. 4D). However, stomachs of mice from indomethacin group showed the number of ulcer spots and red spots (Fig. 4C). Histological examination revealed the stomachs of mice treated with nanocurcumin were almost healthy without any ulcer spots (Fig. 4A), while mice treated with curcumin remained some spots (Fig. 4B). The evaluation was based on the method by Andrade et al. [19], in which ulcers are classified as level III ulcer area > 3 mm^2 [20].

The histopathology of stomach tissues also confirmed the protective effect of nanocurcumin. Fig. 4 showed that stomachs of mice from nanocurcumin group (Fig. 4E) revealed normal gastric mucosa. Meanwhile, the ulcer size did not decrease in the indomethacin group after 22-day treatment and their gastric ulcers were as large as 4.16 mm^2; more extensive damage to the gastric mucosa, edema and hemorrhagic erosion compared with those of control mice. At the same time, as observing mice from curcumin group, although there is no longer gastric ulcer, the stomachs of mice were found with inflammatory cells, mucosal congestion; and cell shedding scatters on their surfaces (Fig. 4F). In the control group, the mice stomach was observed with the damage of the gastric surface epithelial cells and mucosal layer of gastric lumen (Fig. 4H). Treatment with nanocurcumin and curcumin proved comparatively better protection of the gastric mucosa as evidenced by less submucosal edema and reduction in ulcer area. Microscopic examination of stomach sections of mice post-treated with nanocurcumin showed the significant reduction of inflammatory cells and mucosal congestion, and the increase of healthy normal cells in the gastric mucosa, submucosa, serosa, and muscle layers. Total blood protein level in nanocurcumin group was reduced by 2.93 ±0.01 mmol/mg (Table 1), restoring to the normal level (6.59 ± 0.02 mmol/mg) while treatment with curcumin was only marginally less effective with the value of 5.03 ± 0.01 mmol/mg. Result showed the values were significantly different from the control at *p<0.05*. This similar result was also found by Goyal et al. [21] defining the protein level in benzo [a] pyrene induced gastric carcinogenesis.

The gastroprotective effect of nanocurcumin on indomethacin-induced gastric ulcer in mice has been histopathologically screened along with the evaluation of lipid peroxides (LPO), glutathione (GSH), catalase (CAT) and total protein (PRO) concentration in stomachs of all groups of mice (Table 1 and Fig. 5). Mice in group L1 showed a significant increase in the activity of these enzymes CAT (13.15 ± 0.27 mmol/mg), total protein (6.59 ± 0.02 mmol/mg) and the level of GSH (2.78 ± 0.01 nmol/mg); and a significant decrease in the level of LPO (2.20 ± 0.07 nmol/mg) as compared with that of the indomethacin group (non-treatment) with the low value of CAT (5.81 ± 0.53 mmol/mg), total protein (2.93 ±0.01 mmol/mg), GSH (0.49 ± 0.00 nmol/mg) and the high value of LPO (10.89 ± 0.03 nmol/mg). Meanwhile, the curcumin group showed the less effective results with CAT of 8.104f ± 0.265, total protein of 5.03 ± 0.01 mmol/mg, GSH of 1.88 ± 0.00 nmol/mg and LPO of 5.38 ± 0.04 nmol/mg. The results were in agreement with the report of Chattopadhyay et al. [22] who reported that curcumin can effectively block indomethacin-induced increased LPO, thiol depletion, peroxidase inactivation and overproduction of OH to prevent ROS-mediated gastric ulcers.

Malondialdehyde (MDA) is the end-product of polyunsaturated fatty acid, which is often used as indicator for evaluating the lipid peroxidation in gastric mucosal. Lipid peroxidation mediated by oxygen radicals plays a crucial role in the

Table 1. Biochemical analysis of stomach after 22-day treatment

Group	Lipid peroxides nmol/mg	Glutathione nmol/mg	Catalase mmol/mg	Total protein mmol/mg
L1	2.20 ± 0.07^{a}	2.78 ± 0.01^{o}	13.15 ± 0.27^{d}	6.59 ± 0.02^{l}
L2	5.38 ± 0.04^{f}	1.88 ± 0.00^{l}	8.11 ± 0.27^{c}	5.03 ± 0.01^{g}
L3	10.89 ± 0.03^{j}	0.49 ± 0.00^{a}	5.81 ± 0.53^{ab}	2.93 ± 0.01^{a}
L4	4.78 ± 0.10^{e}	1.51 ± 0.01^{g}	12.99 ± 0.27^{d}	6.67 ± 0.00^{l}

Note: Data were expressed as mean ± SD.

Values with different superscripts within the column are significantly different at p<0.05 by Kruskal–wallis test.

L1: The mice administered with nanocurcumin, L2: Curcumin, L3: Indomethacin and L4: Normal mice

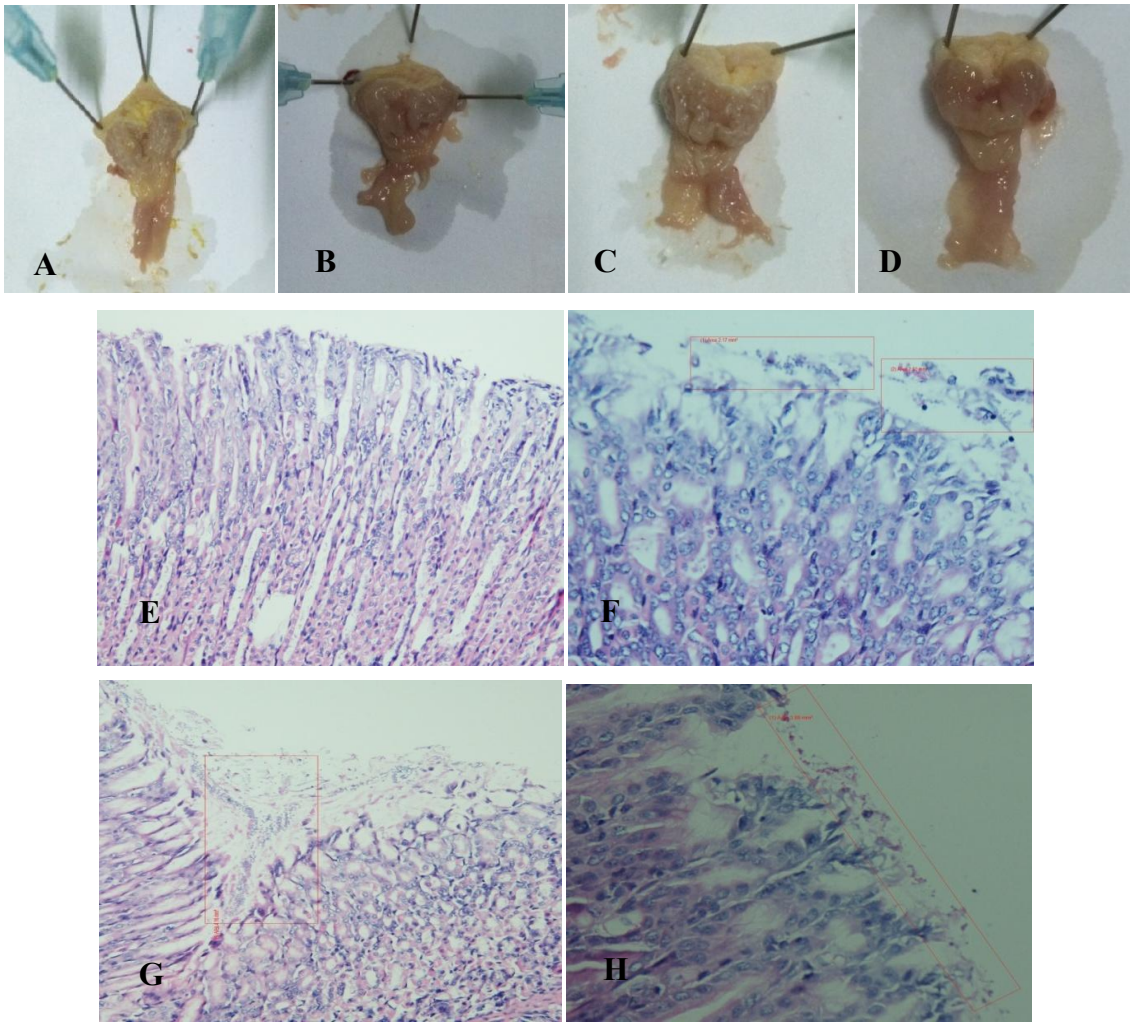

Fig. 4. Images of gastric mucosal injury induced by indomethacin (20 mg/kg, p.o.); the morphology of mice stomach (A) Indomethacin + Nanocurcumin (20 mg/kg), (B) Indomethacin + Curcumin (20 mg/kg), (C) Indomethacin and (D) Control; the microscopy images are at original magnification, x40 from slide sections (E) Indomethacin + Nanocurcumin (20 mg/kg), (F) Indomethacin + Curcumin (20 mg/kg), (G) Indomethacin and (H) Control

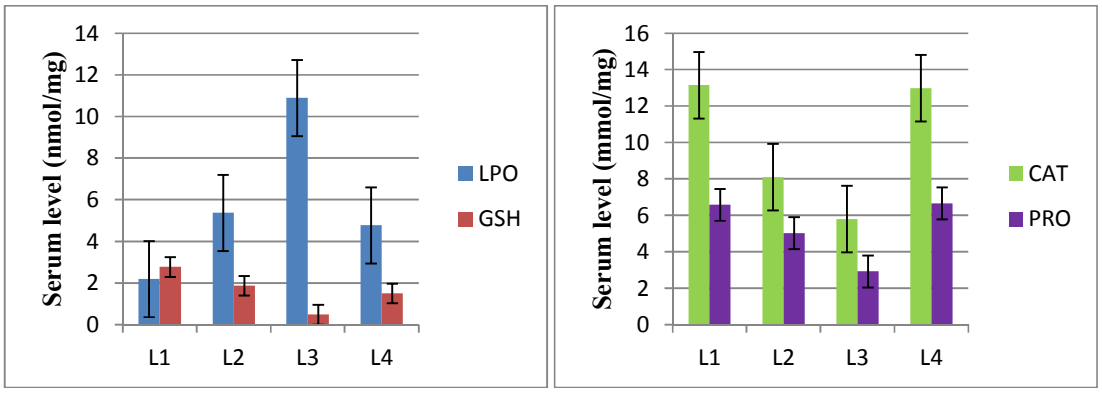

Fig. 5. Effect of nanocurcumin on stomach LPO, GSH, CAT and PRO after 22-day treatment

pathogenesis of the gastric ulceration induced by indomethacin. The measurement of MDA, which is the most abundant of lipid peroxidation products, is a convenient and sensitive method for quantitative estimation of lipid peroxide concentration in many types of samples including drugs, food products and biological tissues from human and animals. Glutathione acts as one of the most important antioxidants in living systems because it is a remover of H_2O_2 lipid peroxides and their products like 4-hydroxinental [21]. GSH activity of stomach in indomethacin group (non-treatment) was found to be lower than in normal group (non-indomethacin induced). The treatment with curcumin, particularly nanocurcumin (20 mg/kg), however, significantly increased GSH activity compared with that of indomethacin group.

Another antioxidant defense system in the body is catalase (CAT). CAT causes direct breakdown of hydrogen peroxide to oxygen and water [23]. Increases in CAT activities were the result of increased H_2O_2 concentrations in mice. The reduction of this protective defense system results in increased sensitivity to free radical-induced cellular damage. The administration of nanocurcumin (20 mg/kg) completely restored the CAT activity to the normal level, indicating the efficacy of nanocurcumin in the recovery of CAT levels against stomach damage induced by indomethacin.

4. CONCLUSION

Nanocurcumin particles were successfully prepared by top down method with the size under 100 nm, and the yield could be reached to15% (w/w) in aqueous solution. The prepared nanocurcumin was found to be significantly effective than curcumin against indomethacin-induced gastric ulcer in mice. These findings provide basis for further pharmacological research that may lead to the development of new drug formulation.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chatterjee A, Chatterjee S, Das S, Saha A, Chattopadhyay S, Bandyopadhyay SK. Ellagic acid facilitates indomethacin-induced gastric ulcer healing via COX-2 up-regulation. Acta biochimica et biophysica Sinica. 2012;44(7):565-576.
2. Jayaprakasha G, Rao LJM, Sakariah K. Chemistry and biological activities of C. longa. Trends in Food Science & Technology. 2005;16(12):533-548.
3. Mei X, Xu D, Wang S, Xu S. Pharmacological researches of curcumin solid dispersions on experimental gastric ulcer. Zhongguo Zhong Yao Za Zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica. 2009;34(22): 2920-2923.
4. Yadav SK, Sah AK, Jha RK, Sah P, Shah DK. Turmeric (curcumin) remedies gastroprotective action. Pharmacognosy Reviews. 2013;7(13):42.
5. Asouri M, Ataee R, Ahmadi AA, Amini A, Moshaei MR. Antioxidant and free radical scavenging activities of curcumin. Asian Journal of Chemistry. 2013;25(13): 7593.
6. Costa da Silva M, Leite MDR, Lima Oliveira SS, Fideles TB, Fook MVL. Morphological evaluation of chitosan/ Curcumin beads and powder: Effect of the methanol as a solvent. Paper presented at the Materials Science Forum. 2016;869: 854-858.
7. Basniwal RK, Buttar HS, Jain VK, Jain N. Curcumin nanoparticles: Preparation, characterization, and antimicrobial study. Journal of Agricultural and Food Chemistry. 2011;59(5):2056-2061.
8. Yang Y, Ong KH. Sustainable formation of curcumin nanoparticle: Stirred tank and confined impinging jet reactor. Journal of Medical and Bioengineering. 2015;4(2).
9. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: Problems and promises. Molecular Pharmaceutics. 2007;4(6):807-818.
10. Parashar UK, Saxena P, Srivastava A. Role of nanomaterials in biotechnology. Digest Journal of Nanomaterials and Biostructures. 2008;3:81-87.
11. Souza MHLP, Lemos HP, Oliveira RB, Cunha FQ. Gastric damage and granulocyte infiltration induced by indomethacin in tumour necrosis factor

receptor 1 (TNF-R1) or inducible nitric oxide synthase (iNOS) deficient mice. Gut. 2004;53(6):791-796.
12. National Academies Press. Guide for the care and use of laboratory animals, 8th Edition. 2010;1-246.
13. Hu L, Kong D, Hu Q, Gao N, Pang S. Evaluation of high-performance curcumin nanocrystals for pulmonary drug delivery both in vitro and vivo. Nanoscale Research Letters. 2015;10(1):381.
14. Loh ZH, Samanta AK, Heng PWS. Overview of milling techniques for improving the solubility of poorly water-soluble drugs. Asian Journal of Pharmaceutical Sciences. 2015;10(4): 255-274.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979;95(2):351-358.
16. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta (BBA)-General Subjects. 1979;582(1):67-78.
17. Aebi H. Catalase *in vitro*. Methods in Enzymology. 1984;105:121-126.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951;193(1): 265-275.
19. Andrade SF, Antoniolli D, Comunello E, Cardoso LG, Carvalho JC, Bastos JK. Antiulcerogenic activity of crude extract, fractions and populnoic acid isolated from Austroplenckia populnea (Celastraceae). Zeitschrift für Naturforschung C. 2006; 61(5-6):329-333.
20. Adinortey MB, Ansah C, Galyuon I, Nyarko A. *In vivo* models used for evaluation of potential antigastroduodenal ulcer agents. Ulcers; 2013.
21. Goyal P, Verma P, Sharma P, Parmar J, Agarwal A. Evaluation of anti-cancer and anti-oxidative potential of Syzygium Cumini against benzo [a] pyrene (BaP) induced gastric carcinogenesis in mice. Asian Pac J Cancer Prev. 2010;11(3):753-758.
22. Chattopadhyay I, Bandyopadhyay U, Biswas K, Maity P, Banerjee RK. Indomethacin inactivates gastric peroxidase to induce reactive-oxygen-mediated gastric mucosal injury and curcumin protects it by preventing peroxidase inactivation and scavenging reactive oxygen. Free radical biology and medicine. 2006;40(8):1397-1408.
23. Higuchi T, Suzuki Y, Fujimura H. Multiple effects of hydrogen peroxide and temperature on antioxidants and bleaching. Paper presented at the Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia (9-13 July); 2012.

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