Evaluation of anti-inflammatory activity and biocompatibility of curcumin loaded mesoporous silica nanoparticles as an oral drug delivery system

OPEN ACCESS
IOP Publishing
Advances in Natural Sciences: Nanoscience and Nanotechnology
Adv. Nat. Sci.: Nanosci. Nanotechnol. **9** (2018) 035007 (9pp)
https://doi.org/10.1088/2043-6254/aad5d5

Evaluation of anti-inflammatory activity and biocompatibility of curcumin loaded mesoporous silica nanoparticles as an oral drug delivery system

Lannie Hadisoewignyo[1], Sandy Budi Hartono[2,3], Angelica Kresnamurti[1], Ivonne Soeliono[1], Yolenta Nataline[1], Gilang Ardi Prakoso[1] and Daeng Agus Rizka Elok Aulia[1]

[1] Faculty of Pharmacy, Widya Mandala Catholic University Surabaya, Kalisari Selatan No.1, Pakuwon City, Surabaya, Indonesia
[2] Department of Chemical Engineering, Widya Mandala Catholic University Surabaya, Kalijudan 37, Surabaya 60114, Indonesia

E-mail: sandy@ukwms.ac.id

Received 14 January 2018
Accepted for publication 28 May 2018
Published 14 August 2018

Abstract

Curcumin, well-known polyphenol drug shows great promise as a therapeutic agent. However, a major concern for this molecule is its low bioavailability. We have recently reported the use of mesoporous silica nanoparticles (MSN) to enhance curcumin bioavailability significantly. In the present work, we investigated anti-inflammatory effects of the curcumin-MSN and related side effects caused to the gastrointestinal tract and kidney, compared with curcumin and diclofenac sodium. The anti-inflammatory effect tests were performed by induction of carrageenan in Wistar rat feet. Ulcerogenic observations were performed macroscopically and microscopically. Kidney histopathology were performed on the average number of necrotic cells of the proximal tubule and distal tubules. There was anti-inflammatory activity in the administration of peroral curcumin and curcumin-MSN, in the absence of significant macroscopic and microscopic changes in the stomach organ. Curcumin-MSN showed a high anti-inflammatory activity comparable to diclofenac sodium but with a significantly enhanced biocompatibility.

Keywords: curcumin, mesoporous silica nanoparticles (MSN), non-steroidal anti-inflammatory drugs, anti-inflammatory effects, kidney histopathology
Classification numbers: 2.05, 4.02, 5.09

1. Introduction

Inflammation is the first response of the immune system to irritation or infection by germs. Inflammation has been considered as a major risk factor for cancer [1, 2]. Patients with inflammation, usually given treatment to slow or limit the process of tissue damage that occurs in the inflammatory area. The inflammatory mediator in the body is a prostaglandin, produced from arachidonic acid, has an essential role in the defence and repair of gastric epithelial cells, produces bicarbonate mucus, inhibits parietal cell secretion, retains mucosal circulation, and restitution of epithelial cells [3].

The commonly used drug classes are non-steroidal anti-inflammatory drugs (NSAIDs). Diclofenac sodium is one of

[3] Author to whom any correspondence should be addressed.

the most used NSAIDs. It was first introduced in the US in the 1990s and has been consumed by more than 1 billion patients. It is also one of the most prescribed NSAIDs [4]. These drugs limit the activity of cyclooxygenase enzymes (COX) thus affects the formation of prostaglandin [1]. There are two types of COX, i.e. COX-1 and COX-2 enzymes [5]. The inhibitory action mechanisms of COX-1 enzymes causes gastrointestinal side effects and also through inhibition of COX-2 enzymes causes cardiovascular side effects. NSAIDs have major side effects on the gastrointestinal tract, particularly gastric irritation leading to the occurrence of peptic ulcers, and adverse reactions to the kidneys [5].

The adverse effects from the use of NSAIDs have intensifying explorations of a natural anti-inflammatory agent with fewer side effects like curcumin. Curcumin is the active ingredient that can be found in the dietary spice turmeric (*Curcuma longa*). There are numerous clinical trial and animal studies that confirm the benefit of curcumin as a therapeutic agent [6]. Curcumin acquires anti-inflammation, anti-oxidant and antiviral properties. It shows great promise for medication of various pro-inflammatory chronic illnesses such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy,cerebral injury, cardiovascular diseases, cancer, allergy, asthma, bronchitis, colitis, rheumatoid arthritis, renal ischemia, psoriasis, diabetes, obesity,depression, fatigue, and AIDS [7, 8].

Research conducted by Anand *et al* [9] suggests that oral administration of curcumin is reported to have low levels of serum and tissue, as well as rapid metabolism and elimination. This is due primarily to the poor solubility of the curcumin. The problem of solubility can be overcome by several solutions such as the manufacture of nanoparticles [10] and the addition of a carrier substance like mesoporous silica nanoparticles [11].

Many researches showed the benefits from using mesoporous silica materials to increase the therapeutical effects of various of drugs namely doxorubicin [12], Paclitaxel [13, 14] and telmisartan (TEL) [15, 16]. These materials have received much attention as a drug delivery system due to its excellent properties such as very large surface area, porous structure, and ease of surface functionalization and biodegradability [16]. These materials also show great promises for oral drug delivery system [15, 17, 18].

Various approaches have been used to develop a drug delivery system of curcumin by using mesoporous silica materials as a carrier. These approaches include the formation of guanidine functionalized PEGylated mesoporous silica nanoparticles (MSN) [19], lipid bilayer-coated curcumin-based MSN [20], curcumin-loaded silica encapsulated porous chitosan [21], mesoporous silica coated curcumin lipid core [22], curcumin silica composites with double functionalization [23] and composite hydrogels of chitosan-MSN [24]. However, many of these synthesis methods require a complex procedure which limits their practical applications. A simple method to form curcumin-mesoporous silica materials is essential.

In addition, most of the studies on curcumin-mesoporous silica materials showed promising *in vitro* test results. The drug delivery systems enhanced curcumin's therapeutic effects. Nevertheless, few *in vivo* study have been done to explore the actual effects of curcumin-mesoporous silica composite. A detailed *in vivo* study on curcumin-mesoporous silica materials is urgently required as proof of concept of the composite's true potential.

Recently we have reported a facile method for the synthesis of mesoporous silica nanoparticles (MSN) as an oral drug carrier to increase the bioavailability of curcumin. Oral administration of curcumin-MSN with an equivalent dose of 10 mg kg^{-1} resulted in curcumin concentration in mice's blood three times higher than administration of curcumin only (free curcumin). The curcumin concentrations from the administration of free curcumin were very low at all-time points of the bioavailability test [25].

In the current study, we used the curcumin-MSN from our previous research to evaluate the anti-inflammatory capability and related biocompatibility. The anti-inflammatory effect of the curcumin-MSN was investigated by using an induction method of carrageenan to white male Wistar rat. The side effects of the curcumin-MSN to digestive and renal tract were studied through microscopic and macroscopic observation of related organs compared to free curcumin and commercial diclofenac sodium (NSAIDs).

2. Experimentals

2.1. Material and methods

Triblock copolymer $EO_{106}PO_{70}EO_{106}$ (Pluronic F127, MW = 13 400), 1,3,5-trimethylbenzene, 3-aminopropyl triethoxysilane, phosphate buffer tablet and Tween 80, diclofenac sodium, carrageenan were purchased from Aldrich. A fluorocarbon surfactant (FC-4) was purchased from Yick-Vic Chemicals & Pharmaceuticals (HK) Ltd. All chemicals were used as received without purification. Curcumin extracts obtained from PT. Javaplant, Solo, Indonesia with 97% purity.

2.2. Animals

The experimental animals used in this study were white male Wistar rats, ages 6–8 weeks weighing 180–220 grams. Prior to use in the research, animals were adapted for one week under the same conditions or treatment, observed by weight weighing and behavioural observations. Animals are considered healthy and may be used for research if they do not show symptoms of illness and weight loss of no more than 10% of initial weight. All animal-related experiments were performed in full compliance to handling protocol of Ethics Committee in Gadjah Mada University, Indonesia (ethics committee approved No. 00094/04/LPPT/III/2017).

2.3. Synthesis of MSN

MSN were synthesized by adding 0.5 g of F127 as a primary surfactant, and also 1.4 g of FC-4 into 60 ml of HCl with a concentration of 0.02 M. The three components were mixed until a clear solution is formed. Then 0.5 g of TMB was added and followed by the addition of 3 g TEOS. The stirring was continued for 24 h at 30 °C. From the stirring process, the solution was removed to an autoclave for the hydrothermal treatment at 100 °C for 24 h. From

the hydrothermal treatment, the product was separated by using a centrifugation method, washed and dried. Finally, a calcination method was used to remove the surfactants. The calcination was performed at 550 °C for 5 h [26].

2.4. Amine functionalization

MSN was modified with 3-aminopropyl triethoxysilane (APTES) to form amine functionalized MSN. MSN was weighed up to 0.6 g then added into 30 ml Toluene. The solution was stirred and heated. After the solution temperature reached 70 °C, then 0.2 g of APTES was added. The stirring was continued for another several hour. Finally, the product was separated by using a centrifugation method and dried [25].

2.5. Curcumin loading

Curcumin-MSN was synthesised by using a rotary evaporator. Curcumin and MSN with ratio 1:4 (50 mg of curcumin and 200 mg of MSN) were mixed in 20 ml of ethanol. The mixture was first sonicated for 15 min. The solution was then heated and evaporated slowly at 55 °C. The process was performed under vacuum condition and continued until the dry curcumin-MSN was obtained [25].

2.6. Anti-inflammatory activity

Wistar rats were fasted for 18 hours. The animals were divided into four groups each of 8. The first group is a control group. The control group (Group-1) was given water for injection (WFI). The second group (Group-2) was given free curcumin with concentration 10 mg kg^{-1}. The third group (Group-3) received curcumin-MSN with concentration 50 mg kg^{-1}. (50 mg curcumin-MSN contains 10 mg curcumin and 40 mg MSN). The last group (Group-4) was given diclofenac sodium with concentration 5 mg kg^{-1}. Inflammatory testing was performed by intraplantar injection of 1% carrageenan on the leg of the rat. Sixty minutes before induced carrageenan, the rats were first treated according to the prescribed group. The edema volume occurring was observed at 30, 60, 120, 180, and 240 min, using a mercury plethysmometer. The percentage of Edema Rate (%ER) and the percentage anti-inflammatory can be determined by using the following equations (1) and (2).

$$\%\mathrm{ER} = \frac{V_t - V_0}{V_0} \times 100\%, \tag{1}$$

$$\text{Percentage of anti-inflammatory} = \frac{\mathrm{AUC}_p}{\mathrm{AUC}_k} \times 100\%. \tag{2}$$

V_t is the rat leg edema volume at time t, V_0 is the initial volume of the rat leg, AUC_k is the area under the curve for the control group, and AUC_p is the area under the curve for the treatment group.

2.7. Ulcerogenic test

Wistar rats were fasted for 18 hours. The animals were divided into four groups each of 8. The first group is a control group. The control group (Group-1) was given water for injection (WFI). The second group (Group-2) was given free curcumin with concentration 10 mg kg^{-1}. The third group (Group-3) received curcumin-MSN with concentration 50 mg kg^{-1} (50 mg curcumin-MSN contains 10 mg curcumin and 40 mg MSN). The last group (Group-4) was given diclofenac sodium with concentration 5 mg kg^{-1}. Treatment was given for seven days, once per day. On the 8th day, animals are sacrificed and exposed on the surgical board, and the animal's stomach is dissected, and the stomach is lifted out. The stomach is opened in a large arch, washed/cleaned with 0.9% NaCl and exposed over the white cork. The ulcerogenic effect was observed with gastric ulcer imaging, number of ulcers were calculated, and ulcer diameter was measured, then compared with the control group. The severity of the ulcer is expressed as the index of ulcer, which is calculated using equation (3)

$$\text{Ulcer index} = A + B. \tag{3}$$

A is average number of ulcer, B is average diameter of ulcer [27]. The index data were analysed statistically, while the histopathology data of gastric ulcers were analysed descriptively.

2.8. Kidney histopathology observation

Wistar rats were fasted for 18 h. The animals were divided into four groups each of 8. The first group is a control group. The control group (Group-1) was given water for injection (WFI). The second group (Group-2) was given free curcumin with concentration 10 mg kg^{-1}. The third group (Group-3) received curcumin-MSN with concentration 50 mg kg^{-1}. (50 mg curcumin-MSN contains 10 mg curcumin and 40 mg MSN). The last group (Group-4) was given diclofenac sodium with concentration 5 mg kg^{-1}. Treatment was given for seven days, once per day. On the 8th day, animals are sacrificed and exposed on the surgical board and then removed the kidney organ, placed in organ pots containing 10% formalin buffer. Hematoxylin-eosin (HE) staining was performed on renal preparations, then renal histopathologic observation, i.e. the number of necrotic cells of the proximal tubes and the distal contrast tubules. Observations were made through five different fields of view in one slice with 1000 times magnification.

2.9. Statistical analysis

Data obtained from each group were analyzed using one-way ANOVA and when data had significant differences between the group of treatments, the analysis was followed by The Post Hoc Duncan test.

3. Results and discussion

In this study, curcumin-MSN with ratio 1 to 4 were prepared similarly to our previous methods. MSN with particle size around 100 nm, the pore size of 10 nm and cubic mesostructure were functionalized with the amine group (APTES) before curcumin loading by using a rotary evaporator [25].

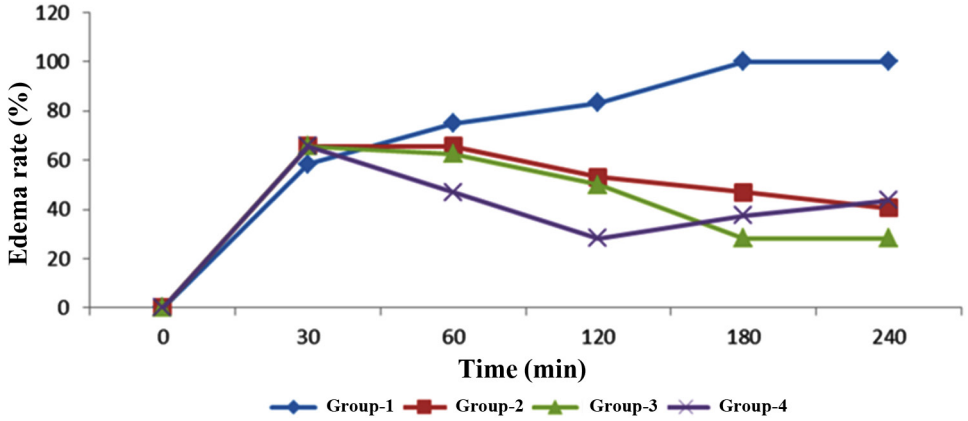

Figure 1. The relationship between the percentages of edema formation rate versus time (minutes). Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg^{-1}.

3.1. Anti-inflammatory activity

The anti-inflammatory test of free curcumin, curcumin-MSN, and diclofenac sodium against edema inhibition in male Wistar rat was demonstrated by comparing the percentage rate of edema formation in rat feet and percentage of anti-inflammatory properties. Figure 1 shows that the edema volume gradually increased and reached a maximum at around 30–60 min for group 2 and 3 and then decreased. Group 4 showed an almost similar trend. It reached a maximum of 30 min and then reduced. At the same period (180–240 min), the curcumin-MSN treatment group (group-3) had the lowest percentage of the rate of formation of edema compared to the free curcumin group and diclofenac sodium.

Figure 1 shows that the rate of edema reached a minimum of three hours for group-3. This result is in agreement with the bioavailability test of curcumin release from MSN that showed a maximum concentration of three hours [25]. Our previous study showed that free curcumin and curcumin MSN had a similar trend. Both showed a maximum concentration of three hours. The concentration of curcumin from curcumin MSN were increased from 0.019 to 0.029 $\mu g\,ml^{-1}$, while concentrations of free curcumin were never above 0.05 $\mu g\,ml^{-1}$. Thus the concentrations of curcumin from curcumin-MSN were 4 to 6 times higher [25]. We believe the concentration of free curcumin was too small to generate the noticeable anti-inflammatory effect. That is why free curcumin did not show similar anti-inflammatory effects as curcumin-MSN.

The maximum concentration caused an optimum inhibition of edema. The value of the calculated area under the curve (AUC_{0-4}) for group-3 and group-4 were quite similar. Table 1 shows curcumin-MSN group (group-3) had the percentage of anti-inflammatory (21.06%), almost the same with group-4 (diclofenac sodium): 23.33%. The lowest percentage was achieved by group-2 (free curcumin), indicating a higher anti-inflammatory curcumin-MSN effect compared with free curcumin.

Figure 1 indicates that curcumin-MSN had a relatively similar anti-inflammatory activity with diclofenac sodium. Curcumin-MSN showed a significantly enhanced performance compared to free curcumin. We believe that this is due to the higher bioavailability of curcumin released from curcumin-MSN compared to free curcumin resulting in higher blood levels and higher anti-inflammatory effects. Free curcumin has a rapid plasma clearance. It is important to make a complex of curcumin with other substance to increase the systemic bioavailability [28].

Curcumin-MSN as an oral drug delivery system increased the bioavailability of curcumin. The bioavailability study showed the curcumin concentration in mice's blood from curcumin-MSN was three times higher compared to free curcumin. During the bioavailability test, it was hard to detect the curcumin concentration from free curcumin [25]. The porous structure of MSN which in nano size limits the particle size of curcumin which influences the solubility. Loading curcumin into the pores of MSN changed the crystalline form into an amorphous structure. These factors synergistically enhanced the bioavailability of curcumin. Also, MSN protects curcumin from harsh acidic environments in the stomach [29].

Curcumin has multiple pathways to suppress inflammatory process. It down-regulates cyclooxygenase-2 (COX-2), lipoxygenase, and inducible nitric oxide synthase (iNOS) enzymes. These conditions inhibit the inflammatory process and tumorigenesis [28]. However, curcumin alone had a limited anti-inflammatory activity due to its low bioavailability. The encapsulation of curcumin into MSN is very important to increase curcumin bioavailability and finally revealed its true potency. Curcumin released from MSN had an equally potent of anti-inflammation compared to diclofenac sodium.

3.2. Ulcerogenic test

3.2.1. Macroscopic observation results in rat's stomach. Macroscopic observation included observation of ulcers in rat stomach by counting the number of ulcers in the stomach. In this study, the results of the index calculation are presented in table 2, and the macroscopic images of the tested animals are shown in figure 2. Based on the calculation of index number of ulcer (table 2), it was found that in the curcumin-MSN group (group-3) the index value of ulcer (2.32 ± 0.82) was almost close to the control group index

Table 1. The value of AUC_{0-4} and percentage of anti-inflammatory.

Group	AUC_{0-4} (μg min ml^{-1})	Anti-inflammatory (%)
1 (WFI)	2.18 ± 0.17	—
2 (Curcumin)	1.82 ± 0.12	16.81
3 (Curcumin-MSN)	1.72 ± 0.07	21.06
4 (Diclofenac sodium)	1.67 ± 0.11	23.33

Note. Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg^{-1}.

Table 2. Ulcer index.

Group	Mean ulcer score		
	Ulcer number	Ulcer diameter (mm)	Ulcer index
1 (WFI)	1.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00
2 (Curcumin)	1.33 ± 0.81	1.33 ± 0.81	2.66 ± 1.62
3 (Curcumin-MSN)	1.16 ± 0.41	1.16 ± 0.41	2.32 ± 0.82
4 (Diclofenac sodium)	3.16 ± 0.41	3.00 ± 0.00	6.16 ± 0.41

Note. Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg^{-1}.

(2.00 ± 0.00). The value of group-3 was lower compared to the group given free curcumin. The largest ulcer index was in the group of diclofenac sodium which was 6.16 ± 0.41. The ulcer index of group-4 was three times higher compared to group-3.The index number shows the severity of ulcers that occur in the stomach.

The control group showed normal or absent gastric mucosal features as shown in figure 2(A). In the administration of 10 mg kg^{-1} free curcumin showed a small number of ulcers (figure 2(B)), whereas on the 50 mg kg^{-1} curcumin-MSN (figure 2(C)) showed even fewer gastric ulcers. Based on observations, ulcers were most common in groups given diclofenac sodium shown in figure 2(D).

The general mechanism of action of NSAIDs in reducing inflammation is by inhibiting the cyclooxygenase enzymes (COX). The inhibition of COX limits the formation of prostaglandin. Diclofenac works stronger against COX-2 compared to COX-1 [30]. It is generally known that the use of diclofenac and other NSAIDs show side effects including cardiovascular, gastrointestinal (GI) and hepatic complications. The inhibition of COX-1 and COX-2 eliminate protection in the gastric mucosa. In addition, one of the factors that might contribute to negative effects of diclofenac is the production of leukotriene. Leukotriene is one of eicosanoid inflammatory mediator. The increase in leukotriene level causes irritation on stomach lining [4]. Administration of diclofenac sodium in rats consecutively for seven days may lead to a much more severe gastric ulcer than the treatment group with curcumin and curcumin-MSN.

Based on research conducted by Srivastava *et al* [31], it showed that curcumin has a more dominant effect on COX-2 than COX-1. The drugs that have a stronger effect on COX-2 than COX-1 or lower ratio COX-2/COX-1 has fewer effects on the stomach and kidney. That is why curcumin-MSN and free curcumin obtained fewer side effects than sodium diclofenac.

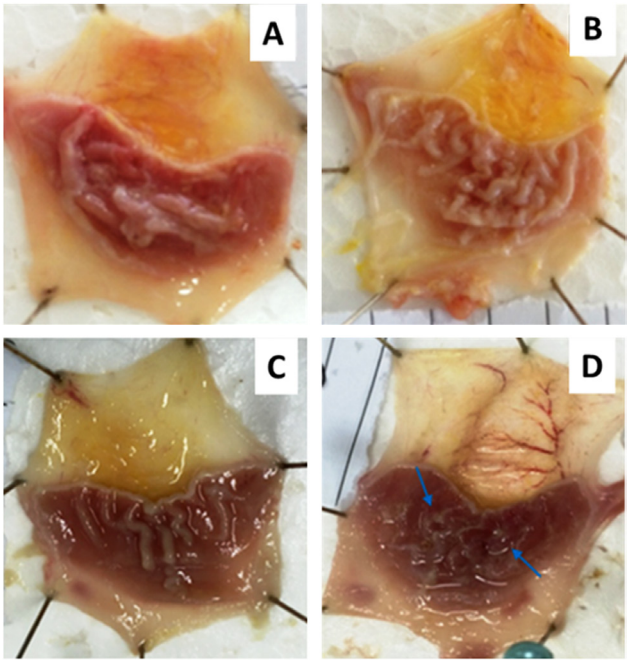

Figure 2. Macroscopic images of rat gastric mucosa. Group-1 (A), Group-2 (B), Group-3 (C) and Group-4 (D). The blue arrows point the ulcer (observed by magnifying glass). Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg^{-1}.

The minimum side effects of curcumin against gastric ulcer due to curcumin's anti-inflammatory properties. It has been shown that curcumin showed therapeutic effects for a various gastrointestinal conditions such as dyspepsia, *Helicobacter pylori* infection, peptic ulcer, irritable bowel syndrome, Crohn's disease, and ulcerative colitis [28]. Research by Pruksunand *et al* [32] showed the efficacy of curcumin in removing ulcer. Phase II of a clinical trial of 45 patients with 25 patients diagnosed with various size of ulcer between 0.5 to 1.5 cm in diameter were given curcumin orally for 12 weeks. The ulcers were disappeared after four weeks in 48% of patients. The other patients required 8–12 weeks to eliminate the ulcer [32].

3.2.2. Microscopic observation of rat gastric. Microscopic observations included descriptive descent on the cross section of rat gastric by looking at specific changes (figure 3). Microscopic observation of necrotic cells in the stomach with 1000 magnification was done by the method of calculating five different fields of view at approximately 100 cells. The results of necrotic cells observation can be seen in table 3. Curcumin-MSN had a very low of an average number of necrotic cells which is very close to the control Group 1. In contrast, sodium diclofenac had the highest number. The number of necrotic cells of group-4 was double than group-3. Figure 3 shows a picture of the defect in the mucosa that penetrates into the submucosa, muscularis propria or deeper.

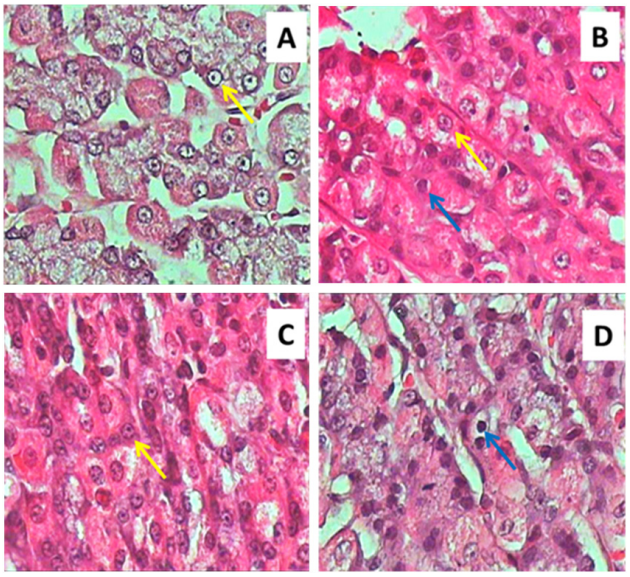

Figure 3. Microscopic images of rat gastric mucosa in the control group and treatment groups were performed in 5 fields of view with 1000 magnification and eosin hematoxylin staining. Group-1 (A), Group-2 (B), Group-3 (C) and Group-4 (D). The yellow arrows point normal cells while the blue ones point necrotic cells. Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg^{-1}.

In the control group showed microscopic images of cells in the gastric mucosa that were almost entirely normal or did not show a specific change and the average number of necrotic cells of 7.30 ± 3.06 is shown in figure 3(A). In the group given 10 mg kg^{-1}, free curcumin showed greater damage compared to the group of curcumin-MSN 50 mg kg^{-1}. The obtained the average number of necrotic cells for curcumin and curcumin-MSN were 10.86 ± 3.97 and 9.46 ± 2.40 respectively, as shown in figures 3(B), (C) and table 3. In comparison, diclofenac sodium with a dose of 5 mg kg^{-1} showed the most cell damage that is 22.76 ± 5.68.

Based on the ANOVA test, it was found that the calculation of the ulcer index on the gastric mucosa for the group given diclofenac sodium was significantly different from the other treatment groups ($F_{\text{count}}(25.698) > F_{\text{table}}(3.10)$). In addition, the mean number of gastric mucosal necrotic cells in the group given diclofenac sodium was also different from that of the other treatment groups ($F_{\text{count}}(19.173) > F_{\text{table}}(3.10)$). From the data above shows that administration of curcumin and curcumin-MSN peroral for seven days had milder side effects compared to diclofenac sodium.

The number of necrotic cells for group-2 and group-3 were quite similar. Both had a low number of necrotic cells which were very close to the control group. In contrast, group-4 had the highest number of necrotic cells. A number of necrotic cells in group-4 were double than that of group-2 and group-3. Many reasons cause necrotic cell death such as trauma, infection, toxins, and others. Necrotic cells are related to the cell swelling and rapid loss of membrane integrity [33, 34]. Necrosis is the death of cells and tissues in the living body.

Table 3. The average number of necrotic cells in rat stomach with five fields of view.

Group	Average number of necrotic cells
1 (WFI)	7.30 ± 3.06
2 (Curcumin)	10.86 ± 3.97
3 (Curcumin-MSN)	9.46 ± 2.40
4 (Diclofenac sodium)	22.76 ± 5.68

Note. Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received Diclofenac sodium 5 mg kg^{-1}.

In necrosis the changes are evident in the cell nucleus with features such as loss of chromatin image, the core becomes wrinkled, not vesicular anymore, looks denser, dark black (pyknosis), the core is divided into fragments. The core is torn (karyorrhexis), the core no longer takes many colours on the colouring because it looks pale, unreal and eventually disappears (karyolysis).

Curcumin with its anti-inflammatory activity has a unique ability to selectively kill cancer cells and not normal cells. Curcumin induces apoptosis of cancer cells but not normal cells [34]. It has been shown that curcumin did not affect normal rat hepatocytes. It showed a high biocompatibility to healthy cells [35].

A recent study showed that MSN is biocompatible and has a low risk of inflammation. It was shown that only mesoporous silica materials with larger particle size (>100 nm) and with a very high dosage caused a higher accumulation in the cells. The accumulation induces a significant release of reactive oxygen species (ROS) and oxidative stress and also an increase of inflammatory genes. The upregulation of the inflammatory genes was mediated through Nuclear factor κB ($\text{NF} - \kappa\text{B}$) and activator protein 1 (AP-1). These conditions promote autophagy's pro activities which ultimately causes necrotic cell death [36]. In this study, we used MSN with particle size around 100 nm. That is why only minor necrotic cells were found.

Research by Çağıltay *et al* studied the effect of NSAIDs on the level of tumour necrosis factor (TNF). They compared diclofenac sodium (100 mg), indomethacin (25 mg) and nabumethon (500 mg). Among these three, diclofenac sodium showed the highest level of TNF [37]. The increase of TNF causes accumulation of reactive oxygen species (ROS) which triggers activation of mitogen-activated protein kinase (MAPK) and disruption of mitochondrial membrane potential which ultimately leads to apoptosis and necrosis [38]. These conditions explain the highest number of necrotic cells from group-4 in Microscopic observation of rat gastric.

3.3. Histopathology test on rat kidney

Group 3 had a very low number of necrotic cells in proximal tubules at 4.83 similar to group 2 (table 4). In contrast, diclofenac sodium caused the highest necrotic cells at 10.67. Figure 4 shows the fundamental differences between normal cells and necrotic cells in the proximal tubule section. In

Table 4. Number of necrotic cells in proximal tubule.

Group	Number of necrotic cells in proximal tubule
1 (WFI)	3.50 ± 1.3
2 (Curcumin)	4.83 ± 0.75
3 (Curcumin-MSN)	4.83 ± 1.94
4 Sodium diclofenac	10.67 ± 4.50

Note. Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received Diclofenac sodium 5 mg kg^{-1}.

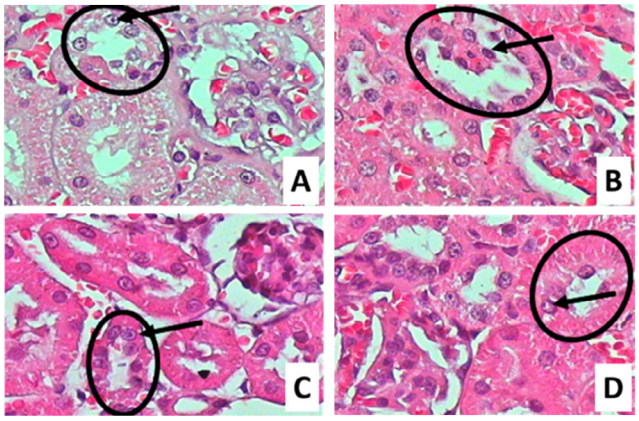

Figure 4. Microscopic overview of the Wistar rats in the proximal tubule section was performed in 5 fields of view with 1000 magnification and eosin hematoxylin staining. Group-1 (A), Group-2 (B), Group-3 (C) and Group-4 (D). Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg^{-1}.

Table 5. Number of necrotic cells on distal tubule.

Group	Number of necrotic cells on distal tubule
1 (WFI)	4.67 ± 1.6
2 (Curcumin)	6.33 ± 3.5
3 (Curcumin-MSN)	7.67 ± 6.28
4 (diclofenac sodium)	9.17 ± 4.62

Note. Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received Diclofenac sodium 5 mg kg^{-1}.

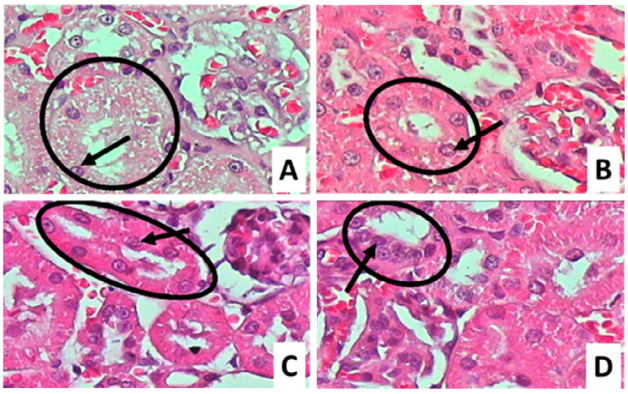

Figure 5. Microscopic overview of the Wistar rats in the distal part of the tubule was performed in 5 fields of view with 1000 magnification and eosin hematoxylin staining. Group-1 (A), Group-2 (B), Group-3 (C) and Group-4 (D). Note: Group-1: (control group) received WFI; Group-2: received curcumin 10 mg kg^{-1}; Group-3: received curcumin-MSN 50 mg kg^{-1} (curcumin 10 mg and MSN 40 mg); Group-4: received diclofenac sodium 5 mg kg^{-1}.

normal cells, the nucleus of the cell is located right in the middle, and the edge of the cell is still intact, while the cells undergoing necrosis or ruptured cells, the cell nucleus is not visible.

Table 5 shows group-3 had an almost similar number of necrotic cells at 7.67 on distal tubule compared to group-2 at 6.33. Group-4 had the highest number of necrotic cells at 9.17. Figure 5 shows the fundamental differences between normal cells and necrotic cells in the distal part of the tubule.

Based on the ANOVA test of necrotic cells observation in the proximal tubule, $F_{\text{count}}\,(9.28) > F_{\text{table}}\,(3.10)$ was obtained, meaning that there was a significant difference between treatment groups. Post Hoc Duncan test $(\alpha = 0.05)$ shows there was no significant difference in the control group, the curcumin extract group and the curcumin-MSN group, but differed significantly with the comparative group (diclofenac sodium), meaning that the curcumin and curcumin-MSN extracts did not cause cellular damage to the renal organ compared with diclofenac sodium. ANOVA test results on necrotic cells observation on distal tubule, obtained $F_{\text{count}}(0.05) < F_{\text{table}}(3.10)$ which means there is no significant difference between treatment groups.

The kidneys are the organs that regulate the internal chemical fluid composition by filtration, reabsorption, and secretion processes. The filtration takes place in the glomerulus, where blood plasma filtrate is formed. In the proximal tubule reabsorption of substances that are useful for the body's metabolism to maintain the internal homeostasis environment, but it also removes the rest of the metabolism from blood to lumen tubules to be removed in the urine. Thus, when the fluid reaches the distal tubule, there are only substances that are not needed by the body to be excreted in the urine. The main target of acute tubular necrosis in cases of poisoning is the proximal tubule of the kidney. Tubule epithelial cells are easily destroyed by contact with toxin materials excreted through the kidneys. These toxin materials are the risk factor of nephrotoxicity. Nephrotoxicity is a drug or toxin-related damage kidney problems [39].

Curcumin can suppress the side effects of toxin molecules that mediate cellular oxidative stress and minimizes induction of ornithine decarboxylase (ODC), finally, removes nephrotoxicity. Okazaki *et al* showed that pretreatment with curcumin reduces the nephrotoxicity of Ferric Nitrilotriacetate (Fe-NTA) (a known renal carcinogen). As a result, necrotic changes can be eliminated thus reduced the histopathological change in mice kidney [40]. Another study by Momeni *et al* also showed that curcumin improves kidney protection against sodium arsenite. Sodium arsenite caused a decrease in the diameter of the glomerulus and proximal tubule, glomerular area. However, for the curcumin-treated group showed no significant different [41]. It can be concluded that curcumin with

its anti-oxidant properties does not cause side effects to the kidney, in fact, it supports kidney protection. In contrast, the use of a combination of NSAIDs enhanced nephrotoxic risk. The delivery of these drugs causes competition for transport protein in the proximal tubular cells. This condition leads to increase of toxin concentration. The accumulation of these substance in cytoplasm causes phospholipid membrane interruption, oxidative stress and mitochondrial injury which ultimately causes proximal tubular necrosis [39].

In general, curcumin-MSN had a better performance of the anti-inflammatory activity and safety features compared to free curcumin. Mesoporous silica nanoparticles not only contributed to the enhancement of curcumin bioavailability but might also support the reduction of inflammation effects. Research by Pelle *et al* showed that silica particle could be used for wound healing in the skin and liver of rats. The absorption of nanoparticles caused tissues adhesion and sealed the wound. After seven days, there was no sign of wound reopening, pathological inflammation and necrosis [42, 43]. Other research by Li *et al* shows the high biocompatibility of curcumin-loaded mesoporous silica incorporated nanofiber mats for hemostasis and anti-bacterial treatment [44].

Compared to other NSAIDs, diclofenac has relatively low-risk factors for GI toxicity and cardiovascular diseases (CVD) [4]. Still, curcumin-MSN had an equally potent in inflammatory activity and fewer side effects compared to diclofenac sodium. Also, unlike other drugs that selectively inhibit COX-2 but causes a higher risk of heart attacks, it has been reported that curcumin shows effects against CVD. Curcumin inhibits NF $-\kappa$B. NF $-\kappa$B is responsible for inducing CRP. CRP is an inflammatory marker and also a risk factor for CVD [45]. This makes curcumin-MSN as a high potent anti-inflammatory agent with a minimum risk compared to others NSAIDs.

4. Conclusion

The administration of peroral curcumin-MSN (50 mg kg^{-1}) caused strong anti-inflammatory activity that is almost similar to diclofenac sodium. The administration free curcumin (10 mg kg^{-1}) and curcumin-MSN (50 mg kg^{-1}) proved to provide no significant macroscopic and microscopic changes to the gastric organ. While giving peroral of diclofenac sodium (5 mg kg^{-1}) showed significant changes in macroscopic and microscopic stomach characterised by the occurrence of gastric ulcers and necrotic cells, suggesting the use of curcumin and curcumin-MSN had fewer side effects compared with administration diclofenac sodium (NSAID group drugs) in the treatment of inflammation. The administration of peroral curcumin extract and curcumin-MSN had a minor effect of necrosis of proximal tubule cells and distal tubule cells. Thus, unlike general NSAIDs that might cause gastro intestine toxicity and related damage kidney problems, curcumin-MSN can be considered as a safe drug. Therefore, we have designed an oral curcumin-MSN drug delivery system which shows a high potency for anti-inflammation with very low side effects. The strong anti-inflammatory properties open the possibility of using this drug for medication of inflammation-related diseases such as cancer, diabetes and many others.

Acknowledgments

We acknowledge the support from Ministry of Research, Technology and Higher Education of the Republic of Indonesia for providing a research grant under the national competition scheme (HIKOM 2017).

References

[1] Ulrich C M, Bigler J and Potter J D 2006 *Nature* **6** 130
[2] Vakkila J and Lotze M T 2004 *Nat. Rev. Immunol.* **4** 641
[3] Konturek S J, Konturek P C, Pawlik T, Sliwowski Z, Ochmański W and Hahn E G 2004 *J. Physiol. Pharmacol.* **55** 5
[4] Gan T J 2010 *Curr. Med. Res. Opin.* **26** 1715
[5] Botting R M 2006 *J. Physiol. Pharmacol.* **57** 113
[6] Heger M *et al* 2017 *Nature* **543** 40
[7] Aggarwal B B and Harikumar K B 2009 *Int. J. Biochem. Cell Biol.* **41** 40
[8] Anand P, Kunnumakkara A B, Newman R A and Aggarwal B B 2007 *Mol. Pharm.* **4** 807
[9] Anand P T S *et al* 2008 *Biochem. Pharmacol.* **76** 1590
[10] Bhawana B R K, Buttar H S, Jain V K and Nidhi Jain N 2011 *J. Agric. Food Chem.* **59** 2056
[11] Jambhrunkar S, Karmakar S, Popat A, Yu M and Yu C 2014 *RSC Adv.* **4** 709
[12] Shahabi S, Döscher S, Bollhorst T, Treccani L, Maas M, Dringen R and Rezwan K 2015 *ACS Appl. Mater. Interfaces* **7** 26880
[13] Wang T, Liu Y and Chao W C 2017 *Nanoscale Res. Lett.* **12** 1
[14] Fu Q, Hargrove D and Lu X 2016 *Nanomedicine* **12** 1951
[15] Yanzhuo Z, Jiancheng W, Xiaoyu B, Tongying J, Qiang Z and Siling W 2012 *Mol. Pharm.* **9** 505
[16] Maleki A, Kettiger H, Schoubben A, Rosenholm J M, Ambrogi V and Hamidi M 2017 *J. Control. Release* **262** 329
[17] Anirudhan T, Sekhar C and Vijayamma A 2017 *Eur. Polym. J.* **93** 572
[18] Tian B, Liu S, Wu S, Lu W, Wang D, Jin L, Hu B, Li K, Wang Z and Quan Z 2017 *Colloids Surf.* B **154** 287
[19] Leila M, Safoora N, Hamidreza K, Sharafaldin M, Mina S, Sonia A, Alireza F and Abbas S 2014 *Eur. J. Med. Chem.* **83** 646
[20] Datz S E H, Schirnding C, Nguyen L and Bein T 2016 *Microporous Mesoporous Mater.* **225** 371
[21] Ahmed A, Hearn J, Abdelmagid W and Zhang H 2012 *J. Mater. Chem.* **22** 25027
[22] Kim S, Diaba R, Joubertb O, Canilhoa N and Pasca A 2016 *Colloids Surf.* B **140** 161
[23] Vishnu S B, Ayan K B, Sujan K M, Sanjiv P, Mariano F, David B, Antonio R D, Chitta R P and Santiago G R 2016 *Mater. Sci. Eng.* C **63** 393
[24] Min Z, Yufang Z, Lingxia Z and Jianlin S 2013 *Sci. Technol. Adv. Mater.* **14** 45005
[25] Sandy B H, Lannie H, Yanan Y, Anand K M, Antaresti and Yu C 2016 *Nanotechnology* **27** 505605
[26] Han Y and Ying J Y 2005 *Angew. Chem., Int. Ed.* **44** 288
[27] Jain N K, Patil C S, Kartasasmita R E, Decker M, Lehmann J and Shrinivas K K 2004 *Drug Dev. Res.* **61** 66
[28] Jurenka J S 2009 *Altern. Med. Rev.* **14** 141
[29] Li L, Liu T, Fu C, Tan L, Meng X and Liu H 2015 *Nanomedicine* **11** 1915
[30] Fosbøl E L *et al* 2009 *Clin. Pharmacol. Ther.* **85** 190

[31] Srivastava K C, Bordia A and Verma S 1995 *Prostag. Leukot Essent Fatty Acids* **52** 223
[32] Prucksunand C, Indrasukhsri B, Leethochawalit M and Hungspreugs K 2001 *Southeast Asian J. Trop. Med Pub. Health.* **32** 208
[33] Rock K L and Kono H 2008 *Annu. Rev. Pathol. Mech. Dis.* **3** 99
[34] Ravindran J, Prasad S and Aggarwal B B 2009 *AAPS J.* **11** 495
[35] Syng-Ai C, Kumari A L and Khar A 2004 *Mol. Cancer Ther.* **3** 1101
[36] Chou C C, Chen W, Hung Y and Mou C Y 2017 *ACS Appl. Mater. Interfaces* **9** 22235
[37] Çağıltay E, Kaplan M, Nalbant S, Akpak Y K, Sahan B and Akmaz I 2015 *Int. J. Res. Med. Sci.* **3** 2280
[38] Sakon S *et al* 2003 *EMBO J.* **22** 3898
[39] Perazella M A 2009 *Clin. J. Am. Soc. Nephrol.* **4** 1275
[40] Okazaki Y, Iqbal M and Okada S 2005 *Biochim. Biophys. Acta* **1740** 357
[41] Momeni H R and Eskandari N 2017 *Exp. Toxicol. Pathol.* **69** 93
[42] Parani M, Lokhande G, Singh A and Gaharwar A K 2016 *ACS Appl. Mater. Interfaces* **8** 10049
[43] Pellé A M, Legrand A, Marcellan A, Luedec L, Letourneur D and Leibler L 2014 *Angew. Chem.* **53** 6369
[44] Li D, Nie W, Chen L, Miao Y, Zhang X, Chen F, Yu B, Ao R, Yu B and He C 2017 *RSC Adv.* **7** 7973
[45] Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Saito T, Manabe I, Imai Y and Nagai R 2006 *Atherosclerosis* **185** 39