Evaluation of Curcumin Nanoparticles against Rabbit Skin Infection

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Abstract: Curcumin is a polyphenolic compound with multiple beneficial effects in human health. It has shown a remarkable anti-inflammatory, antibacterial, antifungal, anticancer, and antioxidant effects. The limitations of curcumin are primarily in its bioavailability. Curcumin nanoparticles is suggested to reduce the limitation of bulk curcumin and probably enhance its desired effect. Curcumin was turned into nanoparticles by using simple wet-milling method. The characterization of curcumin nanoparticles indicated the presence of curcumin in size less than 100 nm. The effect of both curcumin and curcumin nanoparticles on wound healing was investigated in rabbits, in paraffin wax. Rabbits whom under curcumin and curcumin nanoparticles have shown faster healing from day 5 compared to control rabbits whom showed a proximate advancement after day 7. On day 14, rabbits whom treated with bulk and nanosized curcumin have shown a perfect healing with no sign of infection. Furthermore, both bulk and nanosized curcumin have shown a comparable antioxidant effect with ascorbic acid. Furthermore, curcumin nanoparticles were exhibited a slightly powerful antioxidant effect compared to curcumin.

Keywords: Curcumin, wound healing, nanoparticles, inflammation, antioxidant.

1. Introduction

Phytochemicals are compounds found in plants that exist naturally. The utilization of phytochemicals generated from dietary components to battle human diseases, particularly the two most common killers in the developed world, cardiovascular disease and cancer, has piqued public and scientific attention [1].

Curcumin is a compound found in the rhizome of Curcuma longa (L) and other Curcuma species. Curcumin is around 77 percent curcumin in commercial form, with two more similar chemicals, demethoxycurcumin and bis-demethoxycurcumin [2]. These chemicals are classified as diarylheptanoids. Curcuminoid refers to the combination of these three compounds. Curcumin is a crystalline compound that has a bright orange-yellow color to it. Curcumin is a colorant and food ingredient that is widely used. Curcumin as a food additive has an appropriate daily consumption of 0–3 mg/kg, according to the World Health

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Organization (WHO). Its medicinal potential has also been explored, and it is known to have anticancer properties [2-4].
Curcumin, a polyphenol, has been shown to target several signaling molecules while also exhibiting cellular activity, indicating that it has many health benefits [5]. It has been proven to aid with inflammatory disorders [6], metabolic syndrome [7], pain [8], and inflammatory and degenerative eye conditions [9, 10]. It has also been demonstrated to be beneficial to the kidneys [11]. While curcumin appears to have a wide range of therapeutic effects, the majority of these are due to its antioxidant and anti-inflammatory characteristics [5, 6]. Despite its reported anti-inflammatory and antioxidant capabilities, curcumin has a low bioavailability [12], which is thought to be due to inadequate absorption, metabolism, and excretion [13].
When nanotechnology has been introduced to the medical area, the conversion of bulk curcumin into nanosize curcumin has been suggested to improve its qualitative characteristics [14]. Nanoparticle-based drug delivery methods that encapsulate curcumin in liposomes have been shown to solve the difficulties of poor solubility and low bioavailability [15]. We have aimed to produce curcumin nanoparticles in a mechanical approach, and investigate the differences between bulk and nanosized curcumin effects on wound healing in rabbits as an anti-inflammatory agent. Furthermore, the antioxidant activities of bulky curcumin or nanoparticles were a subject of interest in this study.

2. Materials and Methods

2.1. Materials

Curcumin was purchased from Nature's Nutrition (USA), while ascorbic acid, paraffin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), methanol and dichloromethane were purchased from Merck (Germany).

2.2. Preparation of curcumin nanoparticles

The nanosized curcumin was prepared according to the method of Bhawana *et al.* [15]. 0.1g of curcumin powder was dissolved in 20mL CH_2Cl_2 and mixed well for complete dissolving. The curcumin solution was added in a dropwise step into a beaker contained 50mL deionized water, in an ultrasonic device (100 W, 30 KHz), this process was taken a 10 minutes. After that, the beaker was allowed to stand at 27 °C, then stirred for almost 20 minutes, until a clear orange solution was formed. Then the solution was evaporated and an orange powder of curcumin nanoparticles was obtained. The UV-Vis

2.3. Wound healing in rabbits

A paraffin cream was prepared from the bulky curcumin, and from curcumin nanoparticles. 1g of bulk curcumin powder or curcumin nanoparticles powder were mixed with paraffin at 70 °C under continuous stirring for 2h.
Three set of healthy laboratory rabbits were placed in a cage and pre-conditioned for the experiment for 2 days. Each set was contained 5 rabbits, and divided according to the treatment as control set, bulk curcumin set, and curcumin nanoparticles set. The rabbits' dorsal area was circled, and the area was localized with a 10% lidocaine sprayer to produce a wound in a radius of 1.5 cm using a medical blade, leaving the incision open until redness indicated acute inflammation. The rabbits were treated on a daily basis, and observations made during the treatment procedure were recorded. The diameter of wound was measured each day for 14 days, and the results were statistically processed by using ANOVA test.

2.4. In vitro antiradical examination

The activity of bulk and nanosize curcumin to scavenge DPPH was determined in a spectrophotometric method [16]. A series of concentrations (in methanol) each of ascorbic acid, bulk curcumin, and curcumin nanoparticles were prepared (10, 20, 40, 80, and 160 µg/mL). A weight of 0.36 g of DPPH was dissolved in 4mL methanol. 0.15mL of the DPPH solution was mixed with 3mL of each of the prepared concentrations, and with deionized water as control. The tubes were allowed to stand in dark for 30 minutes, then the absorbance of each tube was determine at 517 nm. The activity of each material was calculated from the following equation:
% Activity = $(A_{DPPH} - A_{test}) / A_{DPPH}$

3. Results and Discussion

3.1. Characterization of curcumin nanoparticles

The spectrum obtained from absorbance in UV-Vis for curcumin (Figure 1) indicate the presence of broad peak 430-500 nm, while curcumin nanoparticles have shown a sharp peak around 450 nm. This shift of absorbance band may refer to the formation of curcumin nanoparticles. This was in agreement with the previous studies of Subhan *et al.* (2017) [13], Huong *et al.* (2016) [17], and Shariati *et al.* (2019) [18].

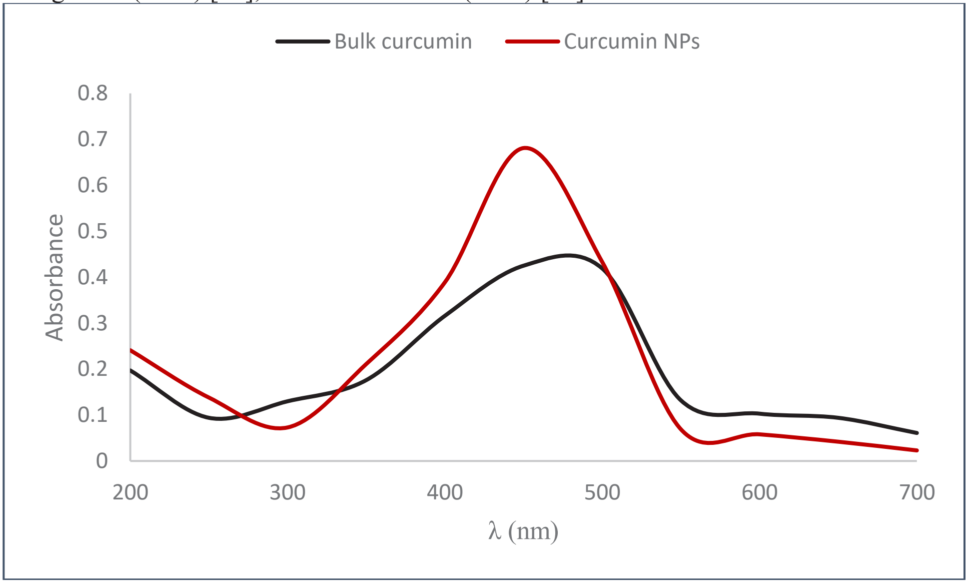

Figure 1: UV-Vis spectrum of curcumin and curcumin nanoparticles.

Curcumin nanoparticles were imaged using a field emission scanning electron microscopy (FESEM) and seemed to be smooth structures with good separation and a spherical form with an average size of 29.75 2.61 nm (Figure 2). This was in line with the findings of previous research [19, 20] using the same method of preparation, as well as their inconsistency with the findings of a study [21] using a different method of preparation.

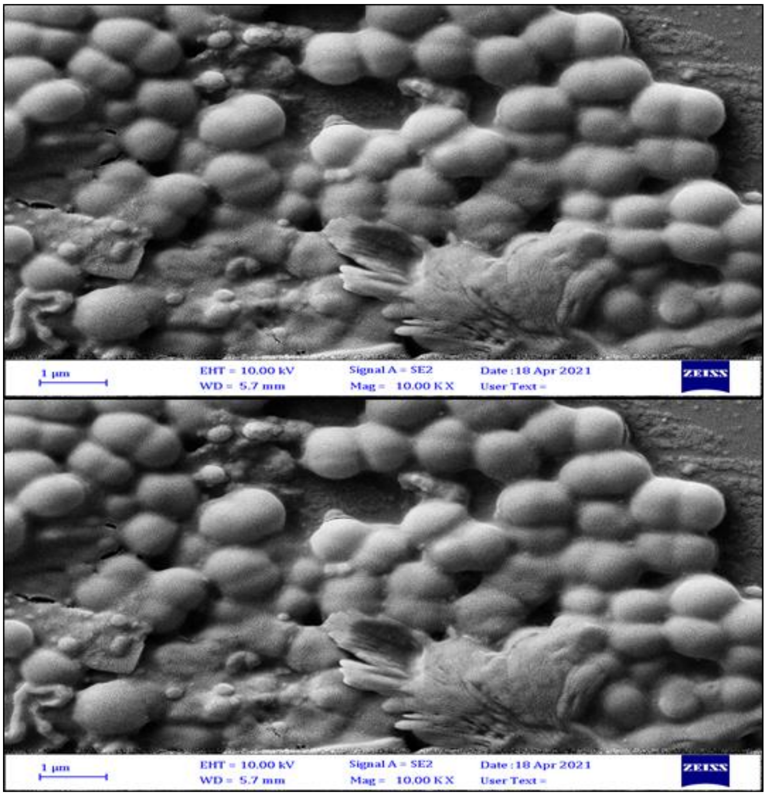

Figure 2: FESEM image of curcumin nanoparticles.

3.2. Wound healing

Table 1: The effect of bulk and nanosized curcumin on wound healing in rabbits.

Healing Period (day)		**Control rabbits**	**curcumin rabbits**	**Curcumin NPs rabbits**	***p*-value**
1st	Diameter (cm)	3.0±0.0	3.0±0.0	3.0±0.0	-
	Observations	Redness	Redness	Redness	
3rd	Diameter (cm)	2.86±0.09	2.82±0.05	2.84±0.06	0.641
	Observations	Redness	Light Redness	Light redness	
5th	Diameter (cm)	2.74±0.06	2.50±0.1	2.50±0.1	0.001*
	Observations	Inflamed area	Clot	Clot	
7th	Diameter (cm)	2.68±0.08	2.10±0.16	2.12±0.16	<0.001*
	Observations	inflammation	Clogged	Clogged	
10th	Diameter (cm)	1.90±0.16	1.38±0.15	1.16±0.15	<0.001*
	Observations	Clogged	Small clot	Small clot	
14th	Diameter (cm)	1.52±0.13	0.56±0.11	0.52±0.11	<0.001*
	Observations	Clogged	Small clot	Small clot	

Mean ± Standard deviation; * Significant at *P* equal or less than 0.05

Table 1 contains the information that obtained from the observations of rabbits post inductive injury. The significant effect of both bulk curcumin cream (2.50±0.10 cm) and curcumin nanoparticles cream (2.50±0.10 cm) were started after 5 days from the treatment compared to control (2.74±0.06 cm). Furthermore, the effect of bulk curcumin cream (0.56±0.11 cm) and curcumin nanoparticles cream (0.52±0.11 cm) was observed to be highly significant ($P<0.01$) after 14th day from injury which have shown complete healing with no sign of inflammation. The differences between bulk and nanosized curcumin was not significant, which may be attributed to the external application of the material, and the good dispersion of both bulk and nanosized curcumin in the paraffin wax. Curcumin paste combined with lime has long been used as a home medicine for inflammation and wound healing [22]. To aid in the restoration of damaged tissue, an optimal wound healing dressing or agent shields the wound tissue from bacterial infection, lowers inflammation, and stimulates cell proliferation [23]. Free radicals are thought to be the main cause of inflammation throughout the wound healing process [24], thus it would be ideal if it also acted as an antioxidant. Curcumin is also involved in tissue remodeling, granulation, tissue creation, and collagen deposition, which has been shown to improve cutaneous wound healing [25].

3.3. Antioxidant activity

The antiradical activity of curcumin and curcumin nanoparticles were registered against DPPH. Ascorbic acid was used as a reference line for the comparison of antioxidant activity between curcumin and nanocurcumin (Figure 3). The results have shown that both curcumin and curcumin nanoparticles were approximate to the antioxidant activity of ascorbic acid in methanol. Nevertheless, the curcumin nanoparticles have shown a slightly more powerful effect against DPPH at the same concentration when compared to curcumin. This may attributed to higher surface area of curcumin nanoparticles which can scavenge more free radicals at a time. The results were in agreement with several previous studies [26-29].

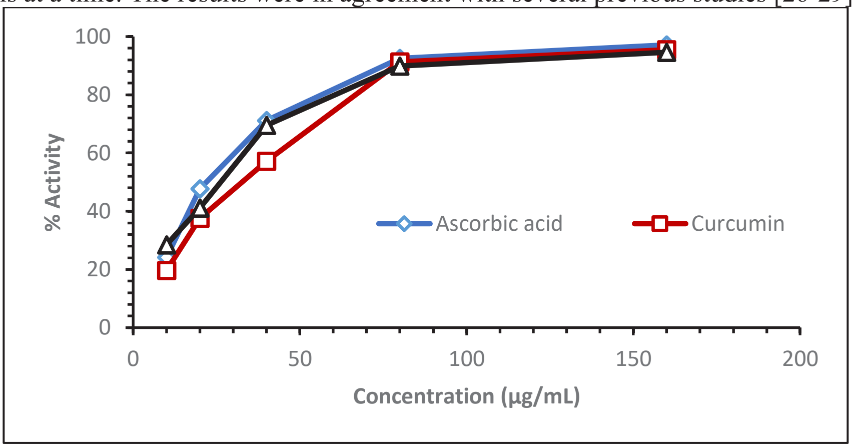

Figure 3: Scavenging activity of curcumin nanoparticles.

4. Conclusions

Curcumin nanoparticles were prepared successfully utilizing a simple method of wet-milling. The uses of curcumin and curcumin nanoparticles as medical agents of wound healing has shown a remarkable activity owing to the anti-inflammatory effect of curcumin with no significant differences obtained in size alterations. As for antioxidant effects, curcumin nanoparticles were slightly more powerful than bulk curcumin, due to the increase of surface area of action.

5. Highlights

Curcumin has multiple health benefits but limited bioavailability. Curcumin nanoparticles were created using wet-milling, promoting faster wound healing and exhibiting potent antioxidant effects, slightly better than bulk curcumin, in a study on rabbits.

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