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In vitro anti-inflammatory activity of silymarin/hydroxyapatite/chitosan nanocomposites and its cytotoxic effect using brine shrimp lethality assay

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ABSTRACT

Silymarin, a bioactive compound, is one of the most prominent drugs used in liver diseases. Chitosan and hydroxyapatite (HAP) are the major materials used employed in many biomedical processes like drug delivery, osteointegration, etc. The nanoparticles and nanocomposites are advanced materials with many biomedical applications in diagnosis and therapeutics. In this study, HAP and chitosan were used as a polymeric material, silymarin as a bioactive compound, and other nanoparticle based combinations. The prepared individual materials and nanocomposites were used for the anti-inflammatory activity and brine shrimp lethality assay. The results clearly show that the nanocomposites are good anti-inflammatory agents with lower toxicity.

Keywords: *anti-inflammatory; chitosan, HAP; nanocomposites, silymarin*

J Popul Ther Clin Pharmacol Vol 28(2):e71–e77; 12 January 2022.

INTRODUCTION

Inflammation is an immunobiological response shown by the body towards infections. The primary symptoms of inflammation are redness, heat, swelling, pain, and even loss of function.[1] They may be acute or chronic that lasts for a shorter or longer duration. The inflammation is indicated by a raised blood flow and vascular permeability with fluid, leukocytes, and cytokine (inflammatory mediators) accumulation in the acute phase and is distinguished by the initiation of specific cellular and humoral immune response to pathogens at the site of tissue injury in the chronic phase. Currently, steroids, nonsteroidal drugs, and even some naturally available products are employed to counteract inflammation by inhibiting the release of cyclooxygenase (COX) enzymes (COX-1 and COX-2), which produce prostaglandins, thromboxane, and other inflammatory mediators.[2,3]

For various decades, conventional medicines have been effectively used for treating several infections.[4] Natural plant-derived compounds have also been used as an alternative medicine since ancient times and hence play an vital role in health care, predominantly in the rural area where access to modern medicine is limited. Traditional drugs derived from plants have been reported to contain phytochemicals (bioactive compounds) that act as defense systems to combat various diseases.[5] Various studies have reported success in validation of traditional plants for their bioactive constituent and its medicinal properties/pharmacological activities for treating many ailments.[6–8]

Silymarin is derived from *Silybum marianum* (milk thistle), an edible plant that has been used medicinally for centuries as an alternative medicine for treating several disorders like liver disease.[9] It's a widely prescribed drug with no side effects. Being native to North America and is also seen to grow in India, China, South America, Africa, and Australia. This natural compound is approved for sale in Canada and many developing countries including India.[10] Silymarin is a polyphenolic flavonoid extracted from the seeds of the milk thistle. The most prevalent component of the silymarin complex is silybin (50–60% of silymarin), which is the most active photochemical and is responsible for the claimed benefit of silymarin. The seeds also contain few considerable amounts of flavonolignans like silychristin, silydianin, taxifolin,[11] betaine, trimethylglycine, and essential fatty acids that contribute to the hepatoprotective and anti-inflammatory effects of silymarin.

The dental implants preferably used to replace missing teeth and restore function are commonly made of titanium, cobalt-chromium alloys, or stainless steel. Titanium has been the most chosen material of choice for the implant. However, there have been several issues regarding the success of the implant and bone tissue osseointegration. So, many implant surface modifications have been made to improve the implant and bone osseointegration which resulted in inflammation. Hence, the present study aims to investigate the anti-inflammatory activity of silymarin, hydroxyapatite (HAP), and chitosan nanoparticles-based nanocomposites.

MATERIALS AND METHODS

Preparation of nanocomposite

The different concentrations of silymarin, chitosan, and HAP were mixed in the beaker and placed on a magnetic stirrer for 48 hours. After the

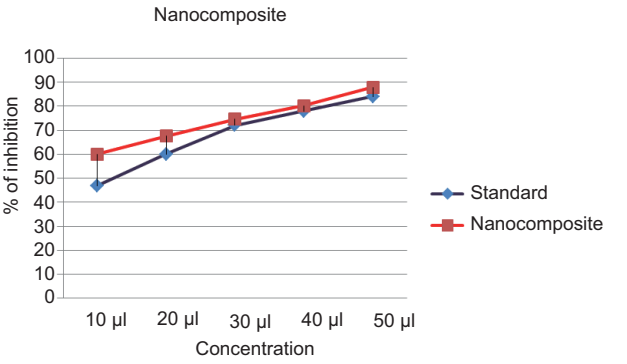

FIGURE 1. Anti-inflammatory activity of nanocomposite**.**

J Popul Ther Clin Pharmacol Vol 28(2):e71–e77; 12 January 2022.

color development, the scanning process was performed using ultraviolet-visible (UV-Vis) spectroscopy from 200 nm to 700 nm.

Anti-inflammatory activity of chitosan, HAP, silymarin, and nanocomposites

Albumin denaturation assay

The anti-inflammatory activity for Chitosan, HAP, silymarin, and nanocomposites was tested by the following procedure with specific alterations.[11,12] Different concentrations of (10–50 µL) nanocomposites were added to 0.45 mL bovine serum albumin (1% aqueous solution), and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid (Figure 1). These samples were incubated at room temperature for 20 minutes and then heated at 55 °C in a water bath for 30 minutes. Later the samples were cooled, and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac sodium was used as the standard, and dimethyl sulfoxide was utilized as the control[14,15]. The percentage of protein denaturation was determined utilizing Equation (1).

$$\%\ \text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (1)$$

Cytotoxic effect of nanocomposites using brine shrimp lethality assay

The cytotoxic effect of the silymarin, chitosan, hydroxyapatite, and nanocomposites were tested by brine shrimp lethality assay using marine water and nauplii eggs.[17]

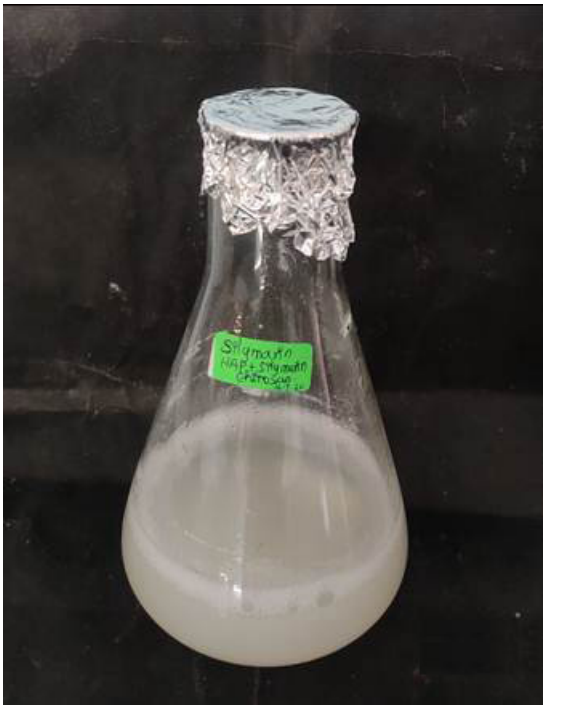

FIGURE 2. Visual observation of formation of silymarin/HAP/chitosan nanocomposites.

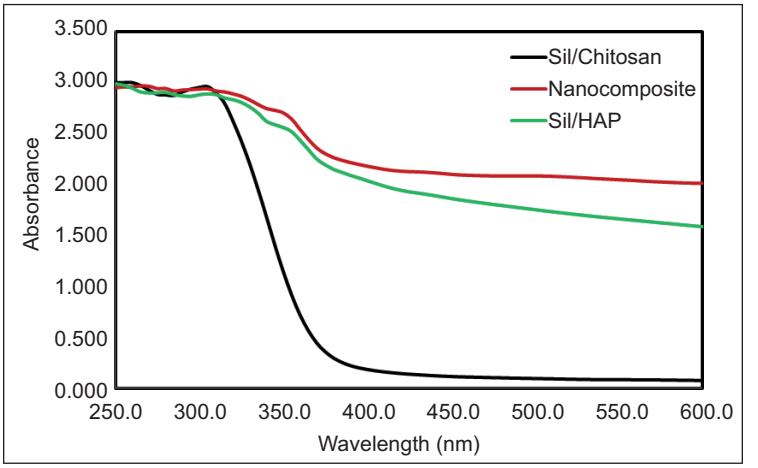

FIGURE 3. Ultraviolet-visible spectroscopy analysis of silymarin/chitosan, silymarin mediated HAP nanoparticles, and silymarin/HAP/chitosan nanocomposites**.**

J Popul Ther Clin Pharmacol Vol 28(2):e71–e77; 12 January 2022.

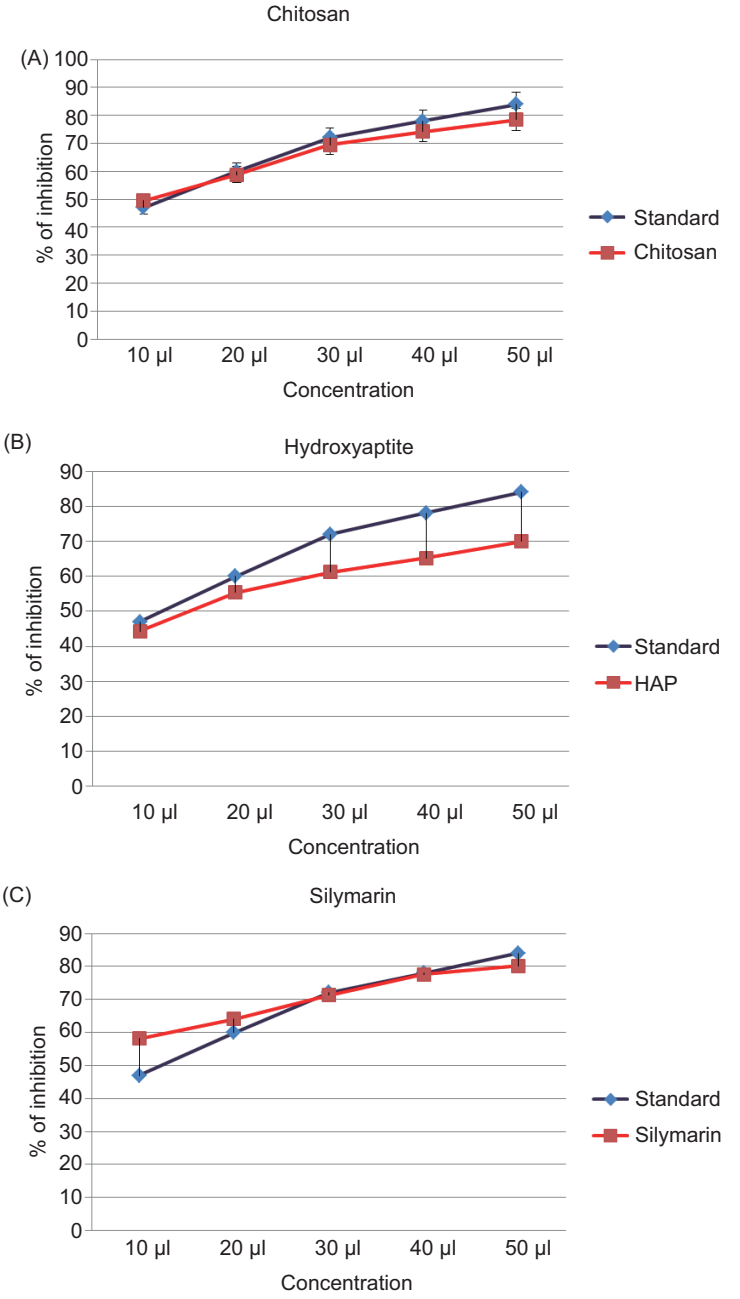

FIGURE 4. Anti-inflammatory activity of chitosan (A), hydroxyapatite (B), and silymarin nanomaterials (C).

J Popul Ther Clin Pharmacol Vol 28(2):e71–e77; 12 January 2022.

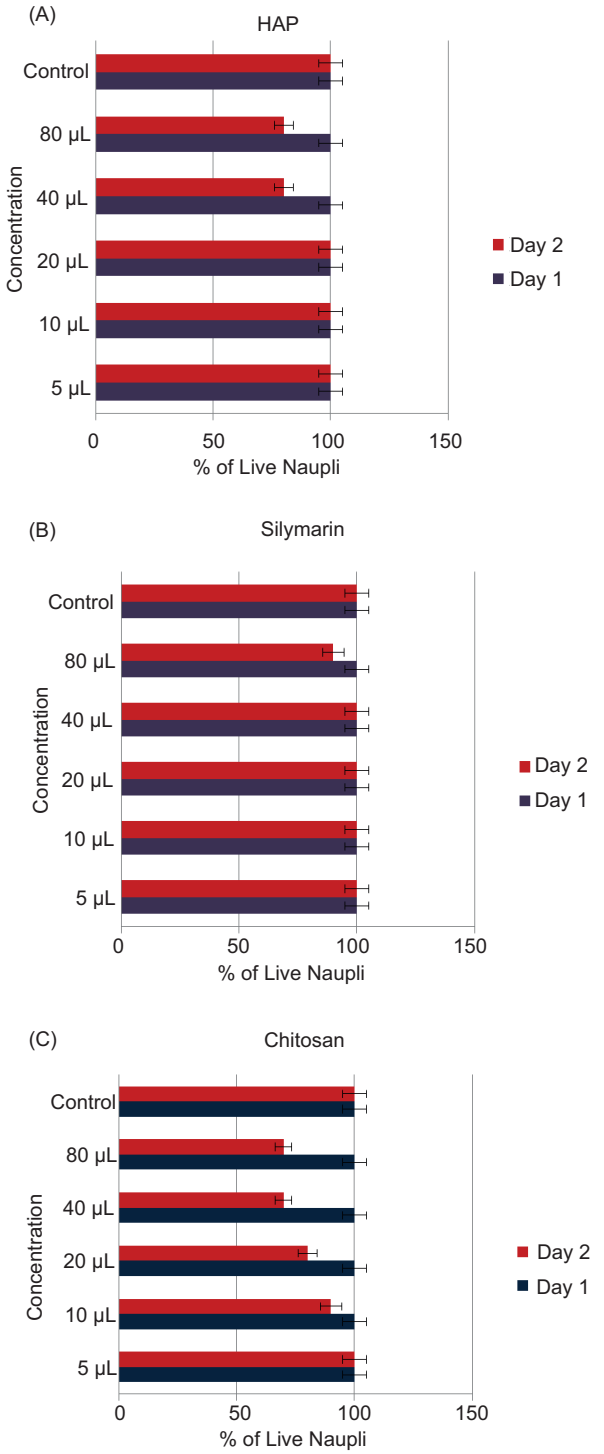

FIGURE 5. Cytotoxic effect of hydroxyapatite (HAP) (A), silymarin (B), and chitosan (C) nanomaterials

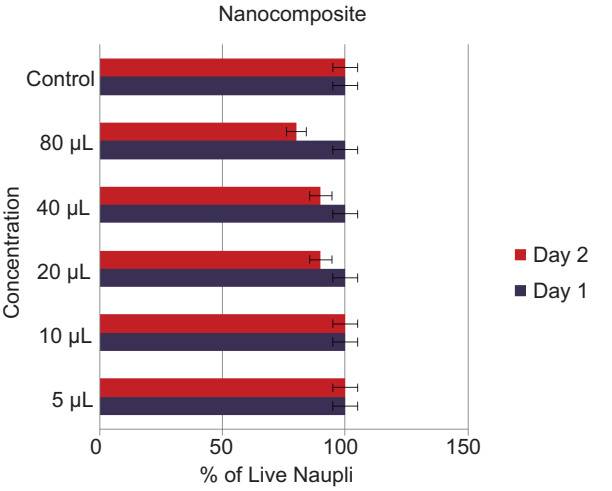

FIGURE 6. Cytotoxic effect of the nanocomposite.

RESULTS AND DISCUSSION

The prepared nanocomposite containing silymarin, HAP, and chitosan is shown in Figure 2. Figure 3 shows the different peaks: silymarin with chitosan is the lowest, silymarin with HAP (middle) shows the peak for mixing of the materials, and the final peak clearly shows the nanocomposite combination with chitosan, silymarin, and HAP.

Figures 3 and 1 clearly show the anti-inflammatory activity of silymarin, chitosan, HAP, and nanocomposites. The bioactive compound silymarin shows the maximum anti-inflammatory activity followed by HAP and chitosan. The three materials nanocomposites show great anti-inflammatory activity, whose values are close to the standard. The nanoparticles are also showing good anti-inflammatory activity.[15, 16]

Cytotoxic effect of the nanocomposites

Figures 5 and 6 show the cytotoxic effect of HAP, silymarin, chitosan materials, and nanocomposites. Silymarin (up to 50 µL) is not toxic to the brine shrimps, and HAP also shows not much toxicity compared with chitosan. But the nanocomposites show minimal toxicity when compared with raw chitosan materials. This result indicates that our

nanocomposite can be used for further biomedical applications.[16,17]

CONCLUSION

In this study three different materials consisting of bioactive compound silymarin, polymer nanoparticles like chitosan, and HAP nanomaterials was prepared. The nanocomposite was a combination of the materials mentioned overhead. The surface plasmon resonance analyzed by using UV-Vis spectroscopy to confirm the formation of the nanoparticles. The percentage of inhibition in anti-inflammatory activity supports the nanomaterial's properties on inflammation and hence can be used for many biomedical applications. The cytotoxicity of nanomaterials and nanocomposites tested against brine shrimps showed minimal lethality.

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J Popul Ther Clin Pharmacol Vol 28(2):e71–e77; 12 January 2022.