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Harnessing the Therapeutic Potential of Curcumin-Anthocyanin photosensitizer in modulating Inflammatory Responses: An in vitro study

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ABSTRACT

Background: Curcumin and anthocyanins are natural compounds with potent anti-inflammatory properties. This study aims to evaluate the anti-inflammatory efficacy of a Curcumin-Anthocyanin photosensitizer gel in an in vitro setting.

Methodology:

The study utilized an in vitro model to assess the anti-inflammatory properties of the Curcumin-Anthocyanin photosensitizer. Anthocyanin was extracted from Punica Granatum, and Curcumin was derived from Curcuma Longa species. These extracts were combined to formulate the photosensitizer gel. The anti-inflammatory activity was measured using the BSA (bovine serum albumin) assay using Diclofenac as a control group.

Results:

The Curcumin-Anthocyanin photosensitizer gel demonstrated significant anti-inflammatory activity in the in vitro models. The BSA assay results indicated a notable decrease in denaturation rates, suggesting effective inhibition of inflammatory mediators in the combination group of about 53%.

Conclusion:

The findings from this study highlight the promising anti-inflammatory effects of the Curcumin-Anthocyanin photosensitizer gel. Its ability to reduce inflammatory mediators and exhibit beneficial photophysical properties, positions it as a potential therapeutic agent for treating inflammatory diseases and as a candidate for further research in photodynamic therapy applications.

Keywords: Curcumin, anthocyanins, inflammatory mediators, photodynamic therapy

INTRODUCTION

Chronic inflammation is a hallmark of numerous diseases, prompting the search for effective anti-inflammatory agents. The intricate interplay between inflammation and chronic diseases has been a focal point of biomedical research which is closely associated with a wide array of pathologies, including cardiovascular diseases [1], malignancies [2], diabetes [3], neurodegenerative disorders [4], and autoimmune diseases [5].

The search for effective anti-inflammatory therapies has led researchers to explore a diverse range of compounds, among which natural products have garnered significant interest due to their potential therapeutic benefits and reduced side effects compared to synthetic drugs [6]. The bioactive plant derivative curcumin is being studied in depth for its strong anti-inflammatory, antioxidant, and anticancer properties [7][8]. Curcumin's rapid metabolizing rate, systemic clearance, and limited bioavailability impede its clinical utilization, despite its great therapeutic potential [9].

On the other hand, anthocyanins, a class of water-soluble pigments found in fruits and vegetables, have also been recognized for their anti-inflammatory and antioxidant activities, influencing various signaling pathways and reducing oxidative stress [10][11]. But like curcumin, anthocyanin's low bioavailability and volatility limit their potential as a medicinal agent.[12].

The combination of curcumin and anthocyanins, leveraging their synergistic effects, presents a novel approach to enhancing their bioavailability and therapeutic efficacy [13]. Recent advancements in drug delivery systems, particularly in the development of photosensitizer gels, have shown promise in improving the stability and bioavailability of these compounds, potentially overcoming the limitations associated with their individual use [14].

Photosensitizers, which are activated upon light exposure, have found applications in photodynamic therapy (PDT), offering a targeted approach in treating various diseases, including microbial infections and cancer, by inducing localized oxidative damage [15][16]. The concept of combining curcumin and anthocyanin in a photosensitizer formulation, aims to harness their anti-inflammatory property and also explores their potentiality in photodynamic applications; opening new avenues for the treatment of inflammatory diseases [17].

Aim of the study

This study seeks to evaluate the anti-inflammatory efficacy of a curcumin-anthocyanin photosensitizer in an in vitro setting, providing insight into its potential as a novel therapeutic agent. This study's primary goal is to evaluate the experimental compound's ability to suppress the denaturation of proteins.

MATERIALS AND METHODS

Study Design

This study employs an in vitro experimental design to investigate the anti-inflammatory effectiveness of a photosensitizer composed of anthocyanin and curcumin extracts.

Study Setting

The experiments were conducted in a controlled laboratory environment within a private institution.

Methodology

Anthocyanin Extraction:

Anthocyanin was extracted from punica granatum (Pomegranate). Pomegranates were peeled out and were dried and powdered. Those peel powder were weighed in an electronic weighing machine. The weighted compound was mixed with methanol and underwent trituration for about 24 hours. Post trituration the mixture was subjected to flash evaporation at 34 degree Celsius. Later filtration was carried out with Whatman filter paper to obtain a pure anthocyanin extract.

Curcumin Extraction

Compound curcumin was extracted from Curcuma Longa Species (Turmeric). Initial dilution of curcumin was carried out with DMSO (Dimethyl sulfoxide) later secondary dilution was done with distilled water. (Figure 1 and 2)

Preparation of Anthocyanin-Curcumin photosensitizer

After the extraction of Anthocyanin and curcumin, molecular weights of the compounds were assessed (Anthocyanin-207.24, Curcumin-368.4) and both the compounds were combined together for better synergistic effect. (Figure 3)

Figure 1 depicts the weighing of peel powder of pomegranate

Figure 2: Methanol dilution of punica granatum

Figure 3 depicts the filtration of tritured compound anthocyanin using Whatman's filter paper post trituration of 24 hours

Figure 4 depicts the process of flash evaporation of anthocyanin at 34 degree Celsius

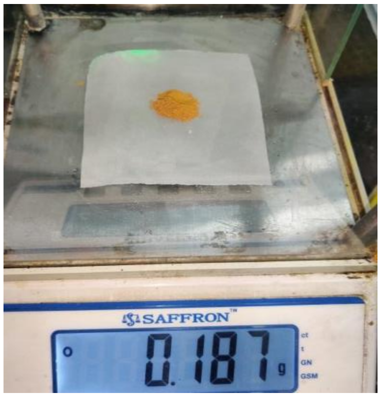

Figure 5 depicts the compound curcumin extracted from Curcuma longa species

Figure 6 depicts the initial dilution of curcuma compound in DMSO (Dimethyl sulfoxide)


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Figure 7 depicts the secondary dilution of compound curcumin in 100 ml of distilled water

Figure 8 depicts the three sample groups consisting of Curcumin, anthocyanin and combination of anthocyanin+curcumin

Bradford protein assay
The test involved preparation of the samples which included,
Control Sample- Diclofenac
Sample 1-Curcumin compound
Sample 2- Anthocyanin compound
Sample 3 -Combination of Curcumin and Anthocyanin dilution of

0.4% BSA (Bovine serum albumin) was prepared in distilled water of pH 6.5. The test solutions (Curcumin, Anthocyanin, Curcumin+Anthocyanin were added to the test tube containing 1ml of 0.4% BSA solution. The solutions were kept in a water bath for 72 °C for 10 minutes and allowed to cool for 30 minutes at room temperature. Diclofenac solution was used as a positive control and assayed in the similar manner. The percentage of protein precipitation was measured at 630 nm.

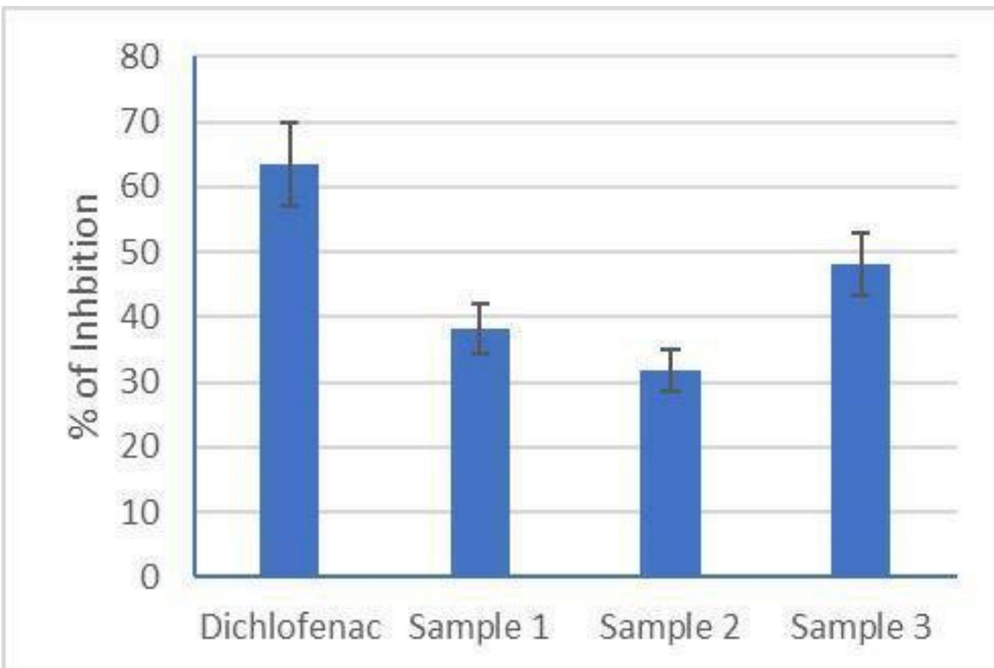
Statistical Analysis

SPSS Software version 23.0 was used to interpret the results statistically according to the level of inhibition in all the four samples. (Figure 4-7)

RESULTS

Repeated measures ANOVA was performed and the most appropriate graph was plotted. The percentage of inhibition for four different samples is plotted as a graph.

The results depicted that Diclofenac showed the highest percentage of inhibition, approximately 70%. Sample 1 containing curcumin showed around 42% of inhibition. Sample 2- anthocyanin showed slightly less inhibition than Sample 1, approximately 37%. Out of all the three samples, Sample 3 (Anthocyanin Curcumin) showed a percentage of inhibition close to Diclofenac (control) which was around 53%. (graph 1)



Graph 1: percentage of inhibition for four different samples

DISCUSSION

The anti-inflammatory efficacy of the Curcumin-Anthocyanin photosensitizer gel, as demonstrated in our study, represents a significant step forward in the quest for natural and effective therapeutic agents. The observed decrease in protein denaturation rates not only indicates a reduction in inflammatory mediators but also suggests the stabilization of cellular structures against inflammatory damage which is a crucial aspect in preventing the progression of chronic diseases [18].

The dual mechanism of action of curcumin and anthocyanins, targeting multiple pathways involved in the inflammatory response, is particularly noteworthy. Curcumin is known to inhibit the NF-κB pathway, a key regulator of inflammation, thereby reducing the expression of pro-inflammatory cytokines [19]. Anthocyanins, has been demonstrated that it can scavenge reactive oxygen species (ROS), reducing oxidative stress, which is a typical component of inflammatory diseases[20]. The combination of these mechanisms in a single photosensitizer gel formulation could explain the enhanced anti-inflammatory effect observed in our study.

Study by Zhi-yun-du et al assessed the anti-inflammatory property of curcumin using BPA assay of which results showed 32% of inhibition [21]. Similarly, the study by urszula et al used DPPH assay and assessed the anti-inflammatory property of anthocyanin which yielded an inhibition ranging from 8.5%-17.4%[22]. These results were in correlation with our current study and furthermore the synergistic potential has a higher inhibition rate which was close to that of the control group about 65%.

Despite the promising results, curcumin's rapid metabolism, low bioavailability, and systemic elimination are challenges that often restrict its therapeutic use that have been extensively documented [23]. Similarly, the therapeutic efficacy of anthocyanins is constrained by their instability and low bioavailability [24]. Our study's approach, leveraging the combination of these compounds in a photosensitizer gel, not only aims to overcome these limitations but also to explore their potential in photodynamic therapy applications, a novel area of research that has shown promise in treating various diseases [25].

Limitations

The limitations of this study includes altered laboratory conditions and processing errors during incubation of the three compounds. In vitro studies would only give an initial exploration of the anti-inflammatory property of the compound rather than a clinical study.

Future scopes

Looking forward, the promising results of this study paves way for further research into the therapeutic applications of the Curcumin-Anthocyanin photosensitizer. Future clinical trials are necessary for validating the safety and efficacy of this innovative formulation.

Conclusion

In conclusion the study highlights promising anti-inflammatory effects of the Curcumin-Anthocyanin photosensitizer as evidenced by notable decrease in protein denaturation rates. This study further emphasizes the synergistic combination of curcumin and anthocyanin as a potential therapeutic agent for treating inflammatory diseases. Additionally, it also positions Curcumin-Anthocyanin photosensitizer as a candidate for further research in photodynamic therapy applications.

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