

Biosynthesis of gold nanoparticles using Caffeoylxanthiazonoside, chemical isolated from *Xanthium strumarium* L. fruit and their Anti-allergic rhinitis effect- a traditional Chinese medicine

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ABSTRACT

This study significantly aims to analyze the effect of CFX coated gold nanoparticles on the allergic rhinitis in mouse model. Female BALB/C mice were intraperitoneally injected with ovalbumin and aluminum hydroxide in order to sensitize on 0th, 7th, 14th, and 21st day. Nano-gold was also nasally inoculated earlier to the intranasal administration of OVA. The acuteness of allergic rhinitis was evaluated based on the nasal indications, interleukin IL-4, and interferon (INF)- γ levels present in the NLF of mice. Also, Hematoxylin-eosin as well as Schiff-periodic acid stain assays were carried out to briefly investigate the histological modifications in the sensitized mice. Nano-gold reduced the incidence of nasal symptoms as well as minimized the IL-4 production, no impact was showed over the levels of INF- γ . Further, the extent of inflammatory cell intrusion was also influenced by the CFX/AuNPs. These experimental findings manifested that AuNPs may considerably inhibit the allergic inflammation in mice models and may be expected to serve as anti-allergic rhinitis agents.

1. Introduction

In the present scenario, the probability of exploring advanced resources in the field of biomedicine has progressively increased due to the budding expansion in the diagnosis and treatment of various cancers by making use of nano-sized composites. Interestingly, noble metal nano-sized particles like platinum (Pt), gold (Au), and silver (Ag) are recently being reckoned as treasured precursors in the design of inventive nano-device systems that are fabricated on the rational and precise incorporation of the functional aspects of the nanoparticles. In particular, surface plasmon resonance, the unique aspect of gold nanoparticles (AuNPs) initiates photo-stability with highly significant light absorptivity [1–4], therefore broadening numerous bio-medicinal applications such as magnetic resonance imaging [5], X-ray computed tomography, drug cancer nanotechnology [6], gene delivery [7], fluorescent labeling [8], and tissue engineering.

Recently, much research attention is being grabbed towards the green synthesis for the reason to generate nontoxic and pure chemicals which are renewable and environment friendly materials. Numerous successful endeavors have been earlier reported for the preparation of nanoparticles by making use of micro-organisms like fungi, yeast, and bacteria [9–12] with enhanced biocompatibility. However, plant extracts are proven to be the most effective approach in nanoparticle

fabrication, for instance, by using broths prepared from garden-fresh plant extracts [13–19]. In particular, AuNPs were earlier reported to be prepared by using various plant extracts such as *Azadirachta indica* [13], *Mentha spicata*, *Ocimum tenuiflorum*, *Hibiscus rosasinensis* [14], *Citrus sinensis* [15], *Maducal longifolia* [16], as well as *Abelmoschus esculentus* [17].

To this point, it was disclosed that the metal salt reduction, which is responsible for the growth of the NPs is achieved by the antioxidant aspects of the plant extracts [13–19]. Generally, plant extracts constitute diverse components, involving active compounds [20,21] that demand efficiency by reciprocally affecting their behavior pharmacokinetically [21–24]. Further, ADMET prophecy, drug-likeness, and in-silico bioactivity [25,26] are helpful in highlighting and predicting the pharmacokinetic aspects of the plant's active constituents. The analysis of allergic rhinitis was earlier reported by using animal models while murine models in particular are suitable to estimate the immunologic process [27,28]. Nano-gold particles are however well-renowned for their anti-microbial activities while their anti-inflammatory properties are not well reported still. In the present research, we have employed allergic rhinitis mouse model with the intent to analyze the influence of nano-gold composites.

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2. Experiments

2.1. Materials

Gold (III) chloride hydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was purchased from the Sigma-Aldrich chemicals and used as received without additional purification. All the reactions were performed using deionized water. Dilute nitric acid HNO_3 was used to wash the glass ware.

2.2. Preparation of Gold Nanoparticles

During the synthesis of AuNPs, 1 mM gold chloride (HAuCl_4) was mixed with 10 mL double-distilled water to obtain gold solution. 10 mL aqueous gold solution was added with 5 mL CFX solution (10 mg/mL) and heated at 80°C for about 10 min till the mixture changes into cherry red in color. This change in color is indicator for the reduction of Au^+ ions to Au^0 nanoparticles. The obtained mixture was stored at room temperature under dark conditions to avoid any further reduction.

2.3. Characterization

JEM-2100EX TEM, HRTEM (Jeol, Tokyo, Japan) was used to evaluate the morphology of the prepared AuNPs. XRD D-MAX 2500/PC (Rigaku, Tokyo, Japan) diffractometer with Cu K ($\lambda = 0.1518 \text{ nm}$) radiation was used to analyze the XRD of the AuNPs. 2800 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) with quartz cell 1 nm resolution was employed to perform the UV-Vis spectral analysis. Pico plus 5500 ILM (Agilent Technologies, USA) AFM was used to perform AFM imaging. The AuNPs were placed onto a (Ruby Mica Co. Ltd., India) Ruby mica muscovite sheet and vacuum dried. AFM JASCO-FTIR, Acoustic alternative current (AAC) spectrophotometer was used to confirm the CFX capping over the surface of the AuNPs.

2.4. Preparation of Animals and Experimental Procedure

Six weeks old female BALB/c infection free mice were collected from Hyosung Science Inc. (Daegu, Korea). All the animals were caged and maintained in laboratory standard conditions under a pathogen-free environment by supplying with food and water as per the Institutional Review Board of Daegu Catholic University Medical Center guidelines. OVA (75 μg) with 200 μL PBS and 2 μg aluminum hydroxide, was intraperitoneally injected to sensitize the mice on 0th, 7th, 14th, and 21st day. After primary sensitization, on 22–30 days, the experimental mice were instilled with OVA 500 μg in 20 μL PBS into their nasal bilateral cavities while the control group was instilled with PBS alone. Gold nanoparticles were dissolved in PBS to treat experimental group while PBS alone is used to treat the control group.

2.5. Evaluation of Nasal Symptoms

The number of nose rubbing movements and sneezing over 15 min duration followed by the last allergen instillation was recorded with respect to the control group.

2.6. Collection of Nasal Lavage Fluid (NLF)

After 24 h following intranasal administration with OVA, NLF was collected. After partial resection of trachea, 18-gauge catheter was used to perform the nasal lavage. Catheter was carefully administered into the opening of trachea in the upward airway direction as well as into nasopharynx. Then, 1 mL cold PBS was perfused into the nasal passages and NLF was collected. The extracted NLF was made to centrifuge for 7 min at 2000 rpm under 4°C and thus obtained supernatant solution was placed at -7°C . Further, ELISA quantization kit was used to measure the quantities of interferon, $\text{INF-}\gamma$ and interleukin, IL-4 in the

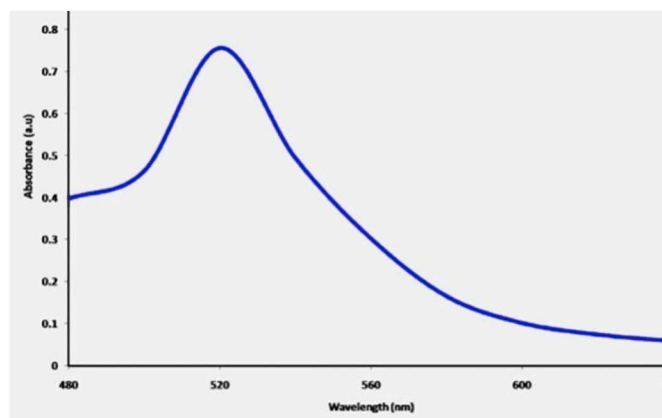


Fig. 1. UV-Visible absorption spectra of the bio-reduced AuNPs.

NLF extract.

2.7. Histological Investigation of Nasal Mucosa

24 h following the intranasal final provocation, the animals were sacrificed by intraperitoneal administration of pentobarbital sodium, lethal dose (120 mg/kg). 10% neutral formalin was used to immerse the decapitated heads for overnight. Then all the parts like eyes, muscle, skin, and mandibles were stripped off from the head. 0.25 mol/L EDTA is used to decalcify all specimens for about 24 h till they turn soft. Fresh razor is used to trim their heads and an excision was made on the anterior nose to brain, apart from nasal sinus. From each mouse, about 3 similar anatomical sections were selected and HE stained for the detailed study. Hematoxylin and eosin stained sections were observed to quantify the inflammatory cell infiltration appearance along with epithelial cell thickness by means of an electron microscope.

3. Result and Discussion

As shown in Fig. 1, UV-vis absorbance showing SPR band is determined to confirm the AuNPs formation in the obtained final reaction mixture UV-vis spectral findings have presented sharp SPR band at about 525 nm, a representative for gold nanoparticle formation [29,30]. This characteristic single sharp peak revealed the occurrence of monodispersed gold nanoparticles [31]. Additionally, this intense peak also referred to the spherical shaped AuNPs, which was further validated by the TEM images [32]. The intense resonance resulted from the exciting surface plasmon vibrations of the bio-reduced gold nanoparticles [33].

Thin film XRD pattern as in Fig. 2, indicated the existence of AuNPs alone. A clear diffraction band at about 38° is usually attributed to facet {111} revealing the face-centered cubic, FCC structure. A major diffraction band at 38.23° of the XRD pattern corresponded to the FCC structure of the AuNPs. However, the other four facets of the diffraction peaks were relatively weaker. In particular, the Bragg diffraction bands at 38.21° , 44.32° , 64.61° , 77.59° and 81.62° showed close agreement with that of the AuNPs.

Transmission electron microscopic images (Fig. 3) have provided extensive information with respect to the morphology and size of the bio-prepared AuNPs. TEM findings have presented that the prepared AuNPs possess various shapes like pentagonal, spherical, and hexagonal and are well-dispersed with about 5–30 nm particle size while the average particle size is 25 nm approximately.

AFM figures of the dispersed CFX loaded AuNPs were shown in Fig. 4A, which presented the closely comparable surface particle thickness. As shown in the 2D-graph (Fig. 4B) AFM, the arbitrary linear zone lying AuNPs (denoted by dotted line) have exhibited different heights in 1–4 nm range. In contrary, surface plane Plasmon peak

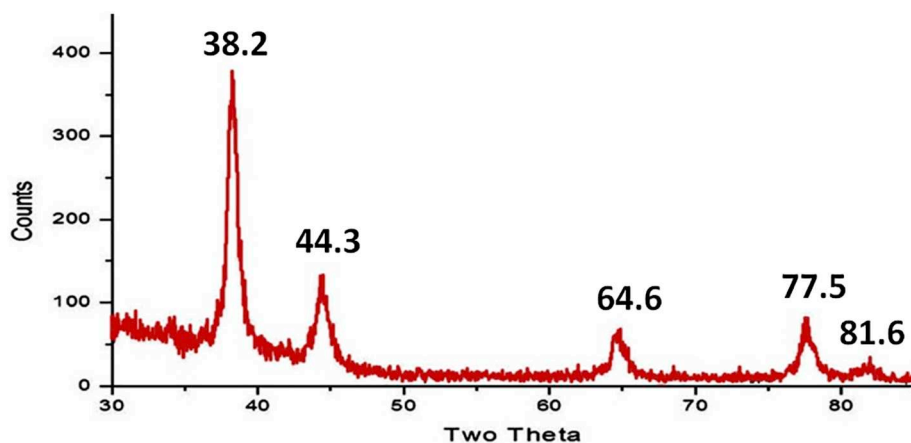


Fig. 2. XRD pattern of bio-reduced AuNPs.

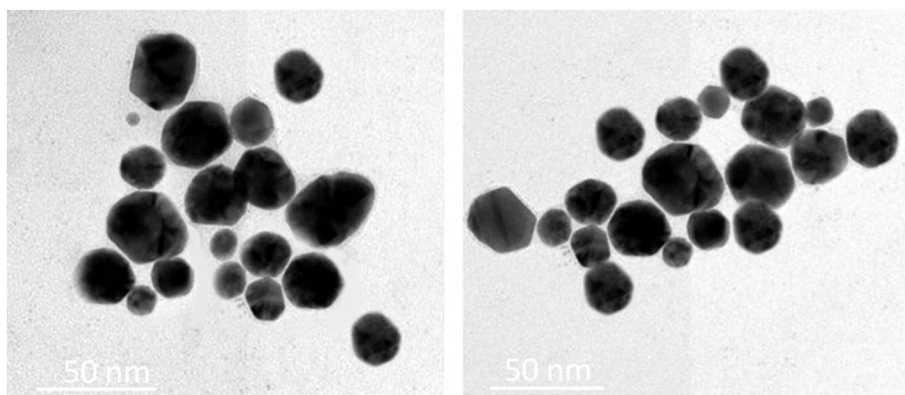


Fig. 3. TEM images of the bio-mediated AuNPs.

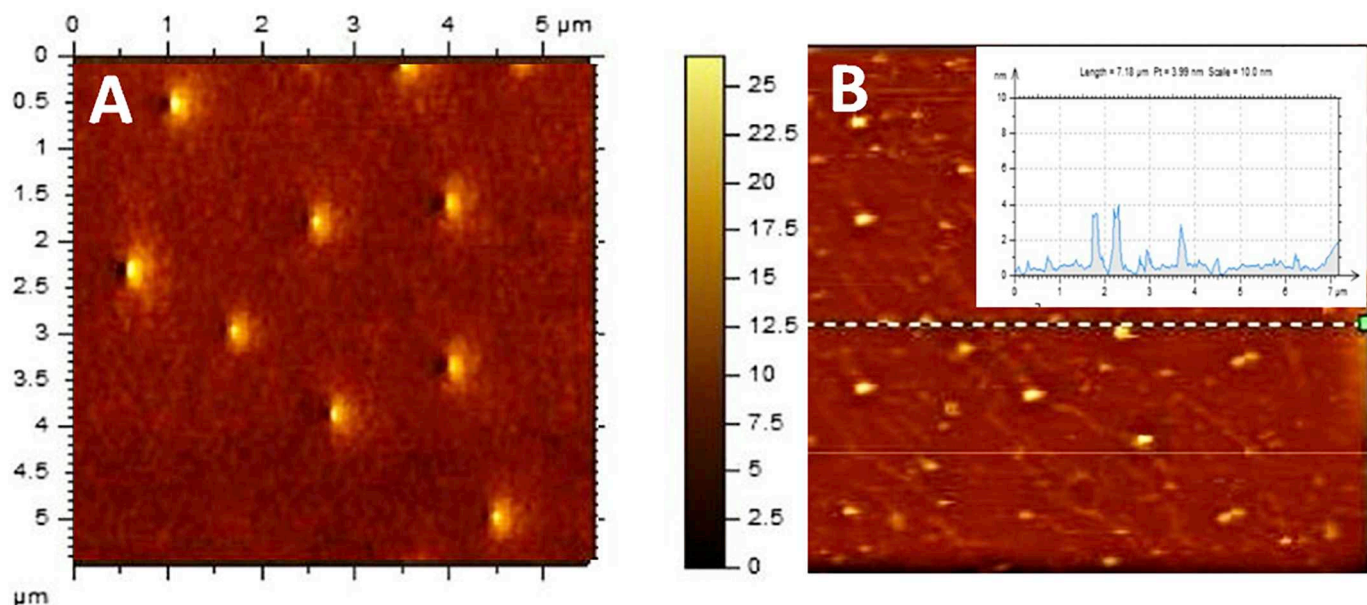


Fig. 4. AFM images and corresponding height profile of bio-fabricated AuNPs.

referred to smaller particle thickness relatively to that of the edge length.

The CFX potential that is accountable for capping and stability of the bio-mediated AuNPs was measured by means of Fourier-transform infrared (FTIR) spectral technique. Fig. 5 displayed the FTIR spectrum of CFX along with its capping over the surface of AuNPs with the

incidence of distinct functional groups. The observed bands at about 3341.1 cm^{-1} and 1742 cm^{-1} corresponded to that of free OH as well as C=O stretching bands respectively. However, bimolecular binding of the AuNPs have shifted these peaks towards 3354 cm^{-1} and 1639.7 cm^{-1} accredited to OH and C=O respectively. The exceptionally strong peak present at 1054 cm^{-1} signified to the CFXC–OH

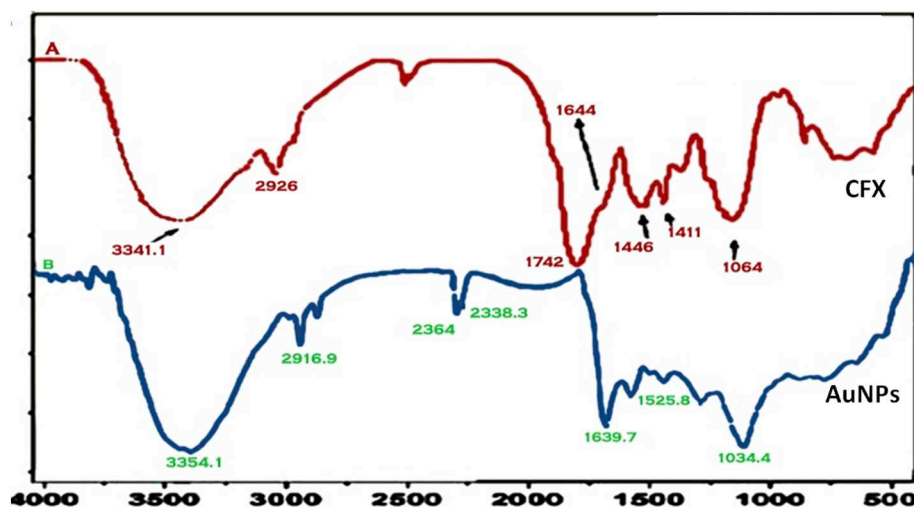


Fig. 5. FTIR spectrum of (A) caffeoylxanthiazonoside and (B) CFX coated AuNPs.

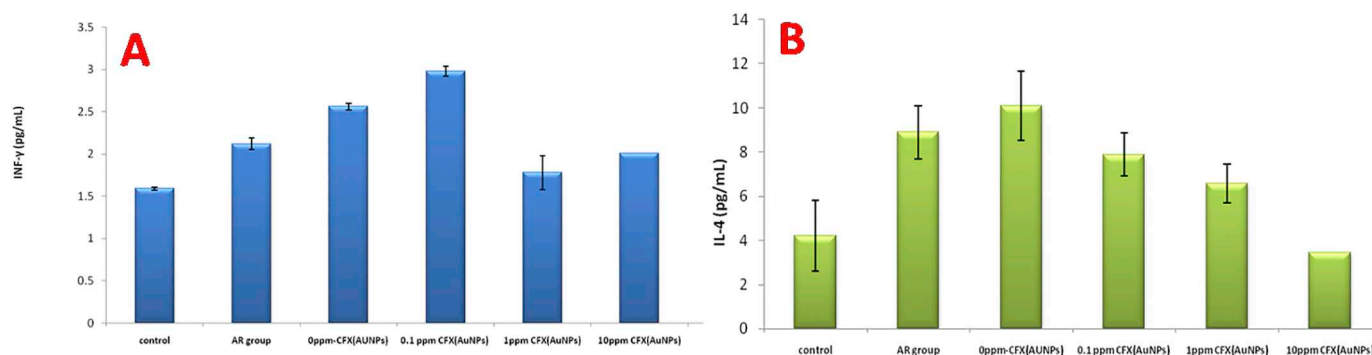


Fig. 6. Graph representing cytokine levels in NLF of each mice model group. (A) Interleukin, IL-4, and (B) Interferon, INF-γ. *P < 0.04.

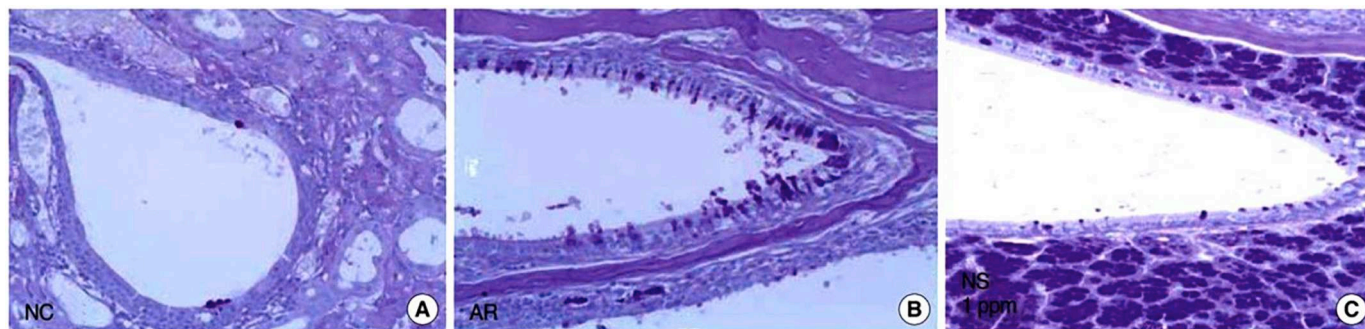


Fig. 7. Images of HE stained nasal mucosa in the (A) Control group, (B) Allergic rhinitis (AR) group and (C) CFX/AuNPs-treated group.

vibration mode, which upon binding to AuNPs shifted towards 1034 cm^{-1} as shown in Fig. 5. The stretching band spotted at about 1644 cm^{-1} represented the free amine, which after capping shifted towards 1525.8 cm^{-1} , specifying the caffeoylxanthiazonoside binding over the surface of AuNPs.

After the final provocation, the number of sneezing and nasal rubbing movements over 15 min time period were recorded as mean behavior score, which was notably 8.3 ± 2.3 in control group, 35.3 ± 3.8 in experimental, AR group, 32.6 ± 3.2 in 0.1 ppm CFX/AuNPs-treated group, 26.4 ± 3.2 in 1 ppm CFX/AuNPs-treated group, and 17.2 ± 1.8 in 10 ppm CFX/AuNPs-treated group respectively. These findings suggested elevated behavior scores in AR group with respect to other groups.

Further, IL-4 levels in NLF were studied as in Fig. 6 and recorded a momentous increase in the AR group, $8.9 \pm 1.6\text{ pg/mL}$ with respect to

the control group, $4.2 \pm 1.2\text{ pg/mL}$. However, production of IL-4 was efficiently hindered by CFX/AuNPs ($P < 0.04$). In contrary, the INF-γ levels in NLF did not show much difference in the AR group, control group, as well as in the CFX/AuNPs-treated group.

From the histological findings as in Fig. 7, it was apparent that the control group has noted less or even no inflammation while the experimental group displayed a raised inflammatory cell intrusion in the submucosal region. From Fig. 8, it was also observed that most of the inflammatory cells are lymphocytes while some of them are eosinophils. In the AR group, an elevation in the degree of inflammatory cell intrusions by 2.5 ± 1.3 was recorded. CFX/AuNPs-treated groups have presented potent inhibition in the inflammatory cell infiltration, as 2.1 ± 0.6 in 0.1 ppm CFX/AuNPs-treated group, 1.8 ± 0.4 with 1 ppm CFX/AuNPs-treated group, and 1.4 ± 0.4 with 10 ppm CFX/AuNPs-treated group; $P < 0.04$. Additionally, the epithelial cell

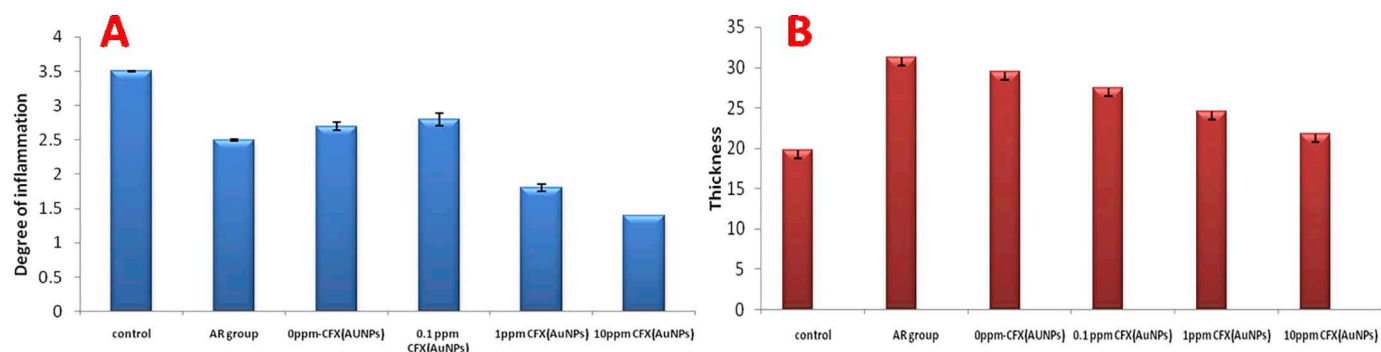


Fig. 8. Graph representing (A) Degree of inflammation and (B) Epithelial cell thickness in the CFX/AuNPs- treated group relative to the AR group and control group. *P < 0.04.

thickness in the nasal mucosa reported a noteworthy increase in the AR group by $31.2 \pm 6.5 \mu\text{m}$ in contrast with the control group by $19.8 \pm 3.1 \mu\text{m}$. Unexpectedly, the, CFX/AuNPs-treated group did not show a substantial influence against epithelial cell thickness.

Experimental findings from the current research validated that CFX/AuNPs instillation have induced allergic symptoms as well as affected the Th2 cytokine (IL-4) levels in NLF fluid. Especially, IL-4 is an anti-inflammatory cytokine generated by monocytes and sometimes by lymphocytes. Th2 influenced cells particularly induce IL-4, IL-5, and IL-13 production to contributed using antibody secretion and humoral response. In this study, CFX/AuNPs showed the ability to stimulate Th2 directed immune-responses, consequently leading to reduced expression of inflammatory cytokines and activation of NF- κ B. In this work, the action mechanism of gold nanoparticles was not explored thus it would be obscure to conclude the ability of CFX/AuNPs to obstruct the NF- κ B mediated activation. In spite of this, our findings suggested that CFX/AuNPs bear the potential to inhibit nasal allergic inflammation via cytokine inhibition from the inflammatory cells.

4. Conclusion

In conclusion, CFX coated AuNPs were efficiently fabricated by using Caffeoxyxanthiazonoside isolated from *Xanthium strumarium* L. fruits extract. Thus obtained AuNPs were characterized to be well-dispersed, pure, face-centered cubic lattice crystals with particle size of about 5–30 nm. Nano-gold particles were found to reduce the inflammatory cell infiltration that was expected to result in increase of nasal symptoms like nasal secretion, sneezing, and rubbing motions in a mouse model. Consequently long-term administration of AuNPs is expected to reduce the nasal mucosa thickness. However, anti-inflammatory pharmacokinetics of CFX/AuNPs has not been completely explained in this work. Th2 cytokine, one of the crucial allergic immune response has been inhibited by the intranasal instillation of CFX/AuNPs. In addition, the inflammatory changes in the nasal mucosa also presented substantial development. Although, further investigation regarding the clinical use is required, it is obviously suggested that gold nanoparticles can be an applicable therapeutic approach in course of their anti-inflammatory property.

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