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Al-Azhar Bulletin of Science: Section A

THE ROLE OF CARBOXYMETHYL CHITOSAN/HYALURONIC ACID EMBEDDED GOLD NANOPARTICLES AS HYPERLIPIDEMIC AND LEPTIN RESISTANCE AGENT

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ABSTRACT

In this work, hyaluronic acid (HA)/carboxymethyl chitosan (CMCTs) blend, embedded with definite amount of gold nanoparticles (AuNPs), were utilized to mitigate lipid profile and leptin resistance in obese rats. AuNPs were produced through Microwave radiation technique, followed by characterization using the state of art analysis; UV and TEM, Average particle size EDX, XRD and IR. Afterward, 70 male albino rats were divided into two main groups: Group one "n=10 rats" fed healthy diet (negative control). The 2nd group fed on the high fructose diet (HFD) for 4 weeks to induce hyperlipidemia and obesity. To this end, the 2nd group is further divided into six sub-groups. The 1st one (G2) received only HFD (positive control), the 2nd subgroups (G3) received HFD+CMCTS+HA, the3rd subgroups (G4) received HFD+ AuNPs, the 4th ,5th and 6th subgroups (G5, G6 and G7) received orally; mixture of both HA and CMCTS in different ratios (3:1; 1:1; 1:3 respectively) embedded all with AuNPs (0.01M), in a dose of 2 mg/kg body weight /day for 4 weeks. The impact of polymers blends with AuNPs on *Methicillin-resistant Staphylococcus aureus* (*MRSA*), *Klebsiella pneumonias* (*K. pneumonia*) and *Candida albicans* (ATCC 10231) (*C. albicans*) was investigated. Results had the best the anti-bacterial and fungal effect for polymers blends in all ratios with AuNPs than for polymers and AuNPs separately. All treated groups (G3, G4, G5, G6 and G7) decreased leptin and lipid profile parameters levels (Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) very low-density lipoprotein (VLDL), atherogenic indices (Atherogenic Index (AI), Atherogenic Coefficient (AC) and Cardiogenic Risk Ratio (CRR)) compared to HFD group. Moreover, the mixture of HA/CMCTS (1:3) adorned AuNPs was more potent than other used ratios in attenuating various biochemical and histological abnormalities resulted due to obesity metabolic disorders. Furthermore, all treated subgroups disclosed normal histopathological heart muscle structures.

Keywords: Carboxymethyl chitosan; Gold nanoparticles; Hyaluronic acid; Leptin resistance; Obesity.

1. Introduction

Biomaterials synthesized from natural polymers are one of the most promising research areas. Hyaluronic acid (HA) is a natural linear polysaccharide made up of repeating units of D-glucuronic acid and N-Acetyl-D-glucosamine disaccharide that was isolated for the first time from the vitreous humor of bovine eyes in 1934 [1]. HA has desired properties in biomedical applications as HA consider a biodegradable, biocompatible, non-immunogenic and nontoxic polymer with hydrophilic properties [2, 3]. Besides, hyaluronic acid contains (-COOH) and (-OH) functional groups that makes it an ideal contender for chemical modification [2, 4]. Carboxymethyl chitosan (CMCTS) as a water-soluble chitosan derivative has a great interest as its applications is expanded, not only hydrophilic properties, but also carboxymethyl chitosan has unique physical, biological and chemical characteristics such as, low toxic, highly viscous, biocompatible, large hydrodynamic volume, and good capability to form fibers, films, and hydrogels[5]. Chitosan and hyaluronic acid jointly can form hydrogels, nanoparticles, microspheres, sponges, and films, with a wide range of applications in biomedical

field [6]. Chitosan with hyaluronic acid could form a new material based on such binary blends.

Gold nanoparticles are used in many fields as favored materials for their unique physical and optical features [7, 8]. Using gold nanoparticles (AuNPs) in many fields is because of its ability to interact with visible light, their electronics and optical features gave them chance to be used in many fields as: bio imaging, medical therapy and drug delivery. They are used as a transcriber in the therapeutic field due to its large surface area/volume ratio, letting their surface coated with various types of molecules including targeting and therapeutics agents [9]. There are numerous approaches for AuNPs production for example (biological, chemical reduction, laser, electrochemical and microwave radiation besides, microwave radiation is a favorable procedure for the production of precise nanoparticles without using a lot of chemicals and in no time [10], nanoparticles obtained by this technique having a great surface area and small size which enable them to infiltrate into the targeted organs [11]. AuNPs could be a new paradigm for weight loss and obesity-related metabolic diseases prevention, as well as a useful research tool for probing biological pathways [12]. In this concern, many studies demonstrated beneficial activities of chitosan as protecting and proliferating agent for reducing hyperlipidemia and stopping impaired lipid metabolism [13-15] . Hyaluronic acid has also been discovered to play a critical role in improving both the electrophysiological and mechanical functions of the heart, as it fixes water in the heart muscle, resulting in improved heart health. As a result, it was recommended that injecting HA into the heart after an infarction might be a viable therapy option [16]. In addition, serum HA has been applied to regulate the sternness of myocardial fibrosis in patients with persistent congestive heart failure [16]. Obesity-related co-morbidities such as high blood pressure, atherosclerosis, impaired glucose tolerance, fatty liver disease, cardiovascular disease, and various cancers killed nearly 3.4 million people (elder than 18 years) during 2016, as per the World Health Organization [17]. Recently consumption of excess quantities of refined carbohydrates and dietary fructose in beverage and food confirmed to rises the risk of dyslipidemia, insulin resistance, heart diseases and obesity [18]. On the other hand, Western foods rich in sugar and saturated fats are major sponsor to worrying rise in obesity and its related illnesses, as these foods have been shown to cause an inflammatory response in the hypothalamus, that increases central leptin resistance and obesity [19]. Leptin as a peptide hormone adjusts food consumption, body mass, and the role of reproduction, as well as playing a fetal role in proinflammatory immunological responses, growth, angiogenesis, and lipolysis. Leptin concentration in plasma and body fat percent have a strong positive relationship [17].

As for, the novelty of this current work is that the two used polymers have a powerful biological effect themselves and used not only as a reducing and stabilizing agents for gold nanoparticles but also in controlling and reducing hyperlipidemia and stopping impaired lipid metabolism of albino rats induced obesity by high fructose diet (HFD) model, through promoting the recapture of lipid profile and leptin hormone level to their normal levels and improving the histopathological changes of the heart muscle that accompanies obesity.

2. Materials and Methods

2.1. Materials

Hyaluronic Acid and Carboxymethyl Chitosan were obtained from Wako Chemicals (USA, Inc.). Fructose was purchased from (Safty Egypt Company Cairo, Egypt). Sigma Co. provided tetra chloroauric acid (HAuCl4), sodium hydroxide "NaOH," and chemicals for the in vitro experiment (USA). Other commonly used diet items were acquired from Morgan Company for Chemicals in Cairo, Egypt (Casein >85% protein, DL-methionine, choline chloride, maize starch, vitamins, minerals, and other items). All used chemicals were with high purity and diet ingredients were food grade with high purity.

2.2. Animals

Seventy healthy mature male albino rats of the "Sprague Dawley strain" weighing (150 ±10g) acquired from (the animal colony, Helwan Farm, Vaccine and Immunity Organization, Cairo, Egypt). Rats were given time to adapt before the experiment began at the Animal House of the Nutritional Chemistry and Metabolism Department, National Nutrition Institute (NNI) - Cairo, Egypt, and were kept at the basic conditions (temperature 22 2 °C, light/dark cycle 12:12 h) and fed a standard diet and freshwater. The experimental animals were kept and cared in agreement with the international rules for the care and usage of laboratory animals, and the experimental protocol was permitted by the national hepatology & tropical medicine research institute (NHTMRI), The General Organization for Teaching Hospitals and Institutes, Cairo, Egypt. (Approval No. A2-2022).

2.3. Microwave Synthesis of Gold Nanoparticles

Gold nanoparticles were produced by microwave technique through using Hyaluronic Acid and Carboxymethyl chitosan mixture with three different ratios of both polymers as (1:3; 1:1;

3:1 respectively). For the produced AuNPs, both HA and CMCTS were utilized as stabilizing and reducing agents. At room temperature, 0.1 g of HA and 0.1 g of CMCTS were dissolved in 100 ml of deionized water. Using very dilute NaOH (0.001 M) drops, the pH was adjusted to 11 for each solution individually, after correcting the pH, each solution ratio was microwaved for 10 seconds, and 2 ml of HAuCl4 (0.01 M) was added to the polymer blend solutions. Each mixture solution was exposed again to microwave radiation for additional 40 s, when time was completed, the color altered from colorless towards deep pink, indicating the establishment of AuNPs. [20].

2.4. Characterization of AuNPs

Over a scanning range of (200–800 nm), Surface Plasmon Resonance (SPR) of AuNPs was evaluated using an absorption spectroscopic technique with a dual beam UV–Vis–NIR spectrophotometer (Unicom UV 500 UV/VIS spectrophotometer). The morphology of the generated AuNPs was imaged using a High-Resolution Transmission Electron Microscope (HR-TEM) (JEOL-JEM 1200) working at a very high voltage of 200 kV (Tecnai G2, FEI, Netherlands). Using well-known software (Image J4s) the size of gold nanoparticles acquired from TEM images was calculated. Identification of nanoparticles was assured by X-ray diffraction (XRD) (D8 Discovery –Bruker Company) via thin film carried out through spin covered instrument, state of measurement was 40 KV and 40 AM (1600W) at speed scan 0.015 and 2theta range from 20° to 80° . The elemental analysis of the polymers samples embedded AuNPs was examined using energy dispersive X-ray (EDX). Fourier transform infrared spectroscopy (FTIR) analysis for the three blended polymers mixtures embedded AuNPs was done using (Perkin-Elmer, FTS1710, Beaconsfeld (UK)).

2.5. Antimicrobial activity study

Antimicrobial activities of the three prepared mixtures of polymers adorned gold nanoparticles, which is prepared as mentioned above was tested on Methicillin-*resistant Staphylococcus aureus* (*MRSA*) as gram positive bacteria, *Klebsiella pneumonias* (*K. pneumonia*) gram negative bacteria and *Candida albicans* (ATCC 10231) (*C. albicans*) as example for fungi. To assess the minimum inhibitory concentration (MIC) a micro dilution technique on micro titer plates (MTP) was used, in which 1: 100 (v/v) of cultures of the tested strains were added up to 200µl of Muller Hinton broth media (MHB media) and dispersed in the wells of MTP with/without compounds. After that, the plates were shaken at 120 rpm for 24 h at 37 °C, and the cell growth was measured at 620 nm using an ELIZA reader (Tecan Elx800, USA). MIC was determined as the lowest concentration of Compounds that inhibit 100% of pathogenic strains in the last wells that did not have any turbidity [21].

2.6. Biological Study

2.6.1. Experimental Design

Food and water were supplied ad-libitum and checked every day for rats which kept individually in wire cages under hygienic and standard conditions. Standard diet presented to rats for 7-days for adaptation after that; they were divided into two main groups. The first group (G1) (n = 10 rats) fed on the healthy basic normal diet (ND) only for eight weeks as a negative control group[22] .The second group (n = 60 animals) fed HFD (fructose 50%) for four weeks to induce obesity and hyperlipidemia [23]. After confirming hyperlipidemia development, rats were divided into six subgroups (n = 10 rats/subgroup). The 1st subgroup (G2) fed on HFD (50%) until the end of the experimental period as an unhealthy control group. The 2nd subgroup (G3) fed HFD and orally injected with HA+CMCTS, the 3rd subgroups (G4) fed HFD and orally injected with AuNPs, the 4th subgroups (G5) fed HFD and orally injected with HA+CMCTS in ratio (3:1) embedded AuNPs , the 5th subgroups (G6) fed HFD and orally injected with HA+CMCTS in ratio (1:1) embedded AuNPs and the 6th subgroups (G7) HA+CMCTS in ratio (1:3) embedded AuNPs in a fixed dose of “2 mg/kg body weight in aqueous solution/day” for all treated groups[20]. Thirty days from launching of the treatment the animals saved fasting for 12 h and weighted then heavily sedated using small ether amount by inspiration; blood was collected from the retro-orbital venous plexus of the eye via capillary tubes in gel activated tubes for the biochemical parameters analysis, samples were centrifuged at (4000 rpm/min) for 10 min by [Gyrozen Low-Speed Centrifuge Model 624R Max. Speed 6000 RPM (110V, 50/60Hz)] centrifuge. Serum was collected and frozen immediately at (-20 °C) till analysis.

2.6.2. Estimation of Biological Parameters

Estimation of body weight (BW) and feed intake (FI) as biological parameters was as follow: Rats weight was measured and recorded weekly then increasing in body weight (BW) of rats in all groups at the end of the experiment represented as a line curve. Daily feed intake (FI) in gram evaluated by the difference between food offered ad libitum- and feed refusals [24].

2.6.3. Estimation of Biochemical Parameters

Serum total cholesterol (TC) and high-density lipoprotein (HDL) were determined according to

Rifai, N [25, 26], serum triglycerides(TG) was assessed according to Young and Friedman [27], the serum low-density lipoprotein (LDL) concentration was calculated according to the following formula: LDL = TC — (HDL + VLDL) and the serum concentration of very low-density lipoprotein (VLDL) was enumerated according to the following formula: VLDL = (TG /5) [28]. Using the given laboratory data, atherogenic indices (Atherogenic Index (AI), Atherogenic Coefficient (AC), and Cardiogenic Risk Ratio (CRR)) were derived as follows: The atherogenic coefficient (AC) was calculated as [(Total Cholesterol (TC) - HDL) / HDL], the cardiogenic risk ratio (CRR) calculated as [TC/HDL], and the atherogenic index (AI) was the log of (TG/HDL) [29]. Leptin Enzyme-Linked Immunosorbent Assay for rats (ELISA) kit (USCN life sciences Inc., Wuhan) was used to determine the levels of leptin in the serum.

2.6.4. Determination of Relative Heart Weight

The animals were sacrificed at the experiment end, then, heart carefully dissected out and weighed in grams (absolute weight). The relative heart weight (RW) for each animal then calculated according to the equation follow : Relative Organ Weight = [Absolute organ weight (g) / Final body weight of rat (g) × 100] [30].

2.6.5. Histopathological examination

Heart prepared for histological examination by rinsing with a saline isotonic solution (0.9% NaCl) to get rid of excess blood, cleaned, fixated at formalin (10%) for 24 h, then dehydrated, cleared and fixed in paraffin wax forming blocks, blocks serially sectioned to four microns thick sections, finally dyed with “hematoxylin and eosin stain (H& E)” to be examined under microscope [31].

2.6.7. Statistical analysis

One-way analysis of variance (ANOVA) was applied to analyse data, afterward Dunnett's multiple comparisons test used. Results presented as mean ± standard division (SD), differences between groups were considered significant when P value (≤ 0.05).

3. Results and Discussion

The current work is pertaining to use two promising naturally occurring polysaccharides; HA and CMCTS, blending in three different ratios, incorporated with AuNPs, on controlling obesity and its complications on albino rats induced obesity with hyperlipidemia by high fructose diet (HFD) model, through promoting the recapture of lipid profile and leptin hormone level to their normal levels and improving the histopathological changes of the heart muscle that accompanies obesity**.**

The production of AuNPs, was performed through safe and eco-friendly method (microwave radiation) which is deemed as an alternate method to the traditional used methods, leads to yield metallic nanoparticles at not worthy short time (less than 60 sec) also, having well précised size distribution, moreover; by using the aforementioned technique it's not necessary to use extra reducing or stabilizing agent [32]. Using of nontoxic materials, HA & CMCTS, reduce the pollution risk for the environment and conducive to new strategies for the green and rapid synthesis of AuNPs [33]. CMCTS and HA were used as attractive reducing and capping agent too for the formed AuNPs. Additionally, their biocompatibility, biodegradability and non-toxicity make them ideal for biomedical applications[34]. CMCTS compared to Chitosan found to be more excellent due to assimilating carboxyl groups, the same for HA that formed of repeated units of disaccharide (N-acetyl D-glucosamine and D-glucuronic acid) that having much carboxyl groups which have the ability to stabilize the formed AuNPs through formation of coordination bond between oxygen of carboxylic groups and Au , beside existing of hydroxyl groups found in both polymer structures, and used to reduce the Au^{+3} to Au^{0} [20]. Microwave-produced radiations interact with the precursors in the reaction scheme, resulting in the creation of reducing energetic species that augment also strengthen the reducibility impact of CMCTS besides HA obviating the need for additional reducing agents and preventing the presence of any toxins sources [35, 36] . Visual observation of the change in the colourless solution of (CMCTS+HA) to deep pink (after adding 2 ml of $HAuCl_4$) as seen in photos of Figure 1 signified the establishment of (AuNPs). Second, the UV–Vis spectrum was used to asses the true wavelength in nm of formative AuNPs. Figure 1 illustrates the UV–Vis absorption of (CMCTS+HA) and polymer-stabilized AuNPs. Samples of (CMCTS+HA) colloidal solution containing AuNPs were scanned from 300 to 750 nm to evaluate the absorption. Few seconds after microwave radiation, transformation from colourless to dark red for the solution containing Au ions because of the establishment of (AuNPs) stabilized by carboxymethyl chitosan and hyaluronic acid as naturally polymers. AuNPs exhibit surface Plasmon Resonance (SPR) absorption in the "520–580 nm" range according to published studies [37] . Because of AuNPs' SPR absorption, the aforementioned created AuNPs in this study have a distinct and well-defined absorption band (523 nm) The AuNPs blend was supported by the occurrence of an SPR peak in this range, as well as the reaction mixture's synchronised colour shift [32, 38]. TEM images; Figure 2 (a-f) illustrated that, AuNPs created had a

spherical dimension that was tiny (less than 7 nm) with good distribution that showing the perfect function of CMCTS and HA polymers with microwave radiations in reducing and précising the size of nanoparticles formed.

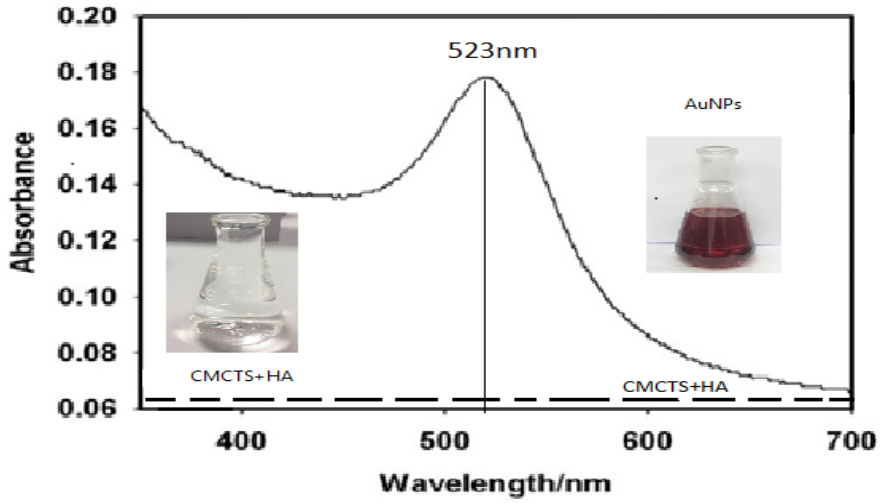

Figure 1. UV-Vis of (CMCTS+HA) Mixture and AuNPs stabilized by polymers mixture.

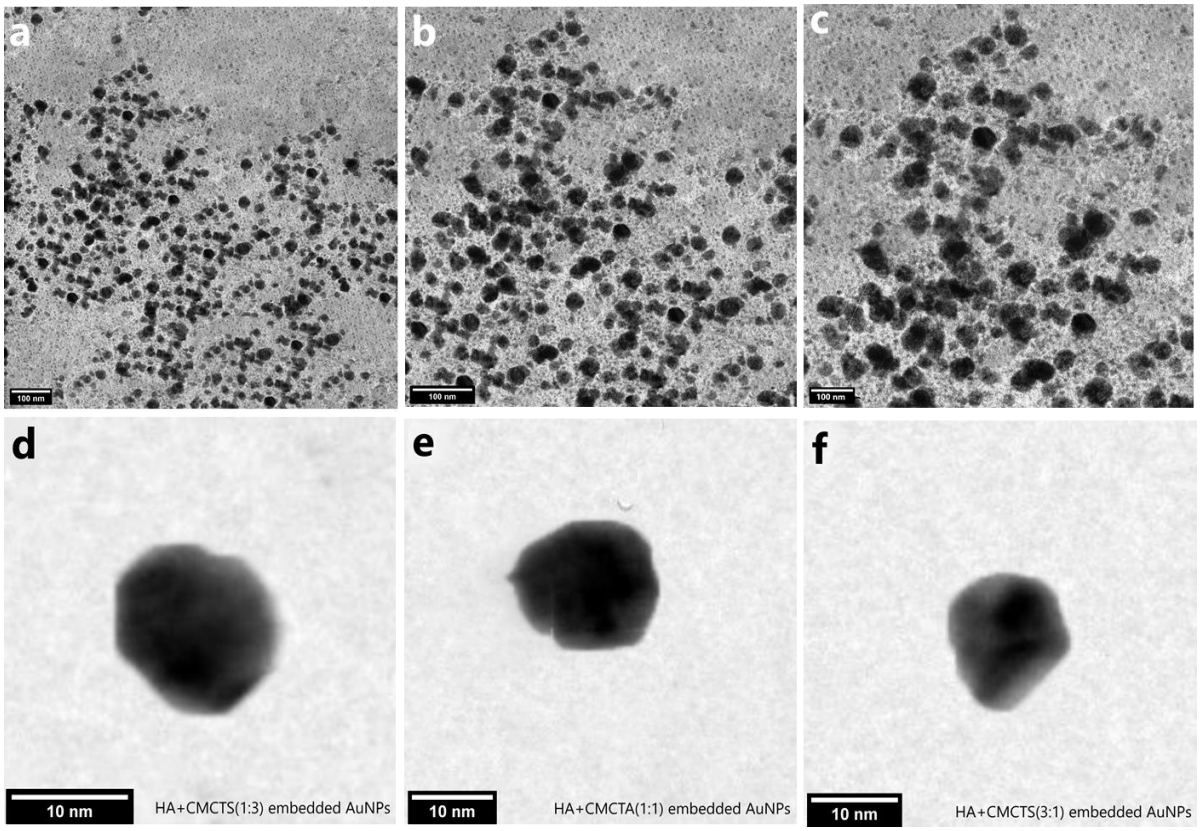

Figure 2. High-resolution TEM (HR-TEM) images of all polymer mixtures embedded AuNPs, where:(a ,d) TEM image for mixture of HA+CMCTS (1:3) embedded AuNPs in scales (100,10) , (b, e) TEM image for mixture of HA+CMCTS (1:1) embedded AuNPs in scales(100,10) and (c, f) TEM image for mixture of HA+CMCTS (3:1) embedded AuNPs in scales (100,10).

Figure 3 a, b and c reveals the hydrodynamic size, X-ray diffraction (XRD) and energy dispersive X-ray (EDX) of AuNPs respectively. The acquired, diagram in Fig. 3a reveals that, the average size for synthesized AuNPs distribution assessed using dynamic light scattering (DLS) technique, which was the most prevalent and common method for defining the size distribution outline of nanoparticles [39]. The polydispersity index showed that the average size was 4.18 nm

(0.03). The resulted size is extremely small, demonstrating the ability of both the microwave and the polymer mixture (HA+CMCTS) to simultaneously reduce all Au ions and control the size to a precise minor diameter. Additionally, these nanoparticles are stabilized by powerful stabilizing agents HA and CMCTS, which cap the particles and stop them from aggregating. Therefore, the PDI value (0.03) is regarded as a good value for the prediction of the disparity as well as the homogeny of the produced colloidal solution of AuNPs, which is consistent with TEM images in terms of the distributed particles or homogeneity of the particles in hand. XRD has been used to highlight the crystallinity characteristics of nanoparticles in order to determine the purity of the produced AuNPs. Per the ICCD database (COD 9008463), the graph has distinct, strong four peaks at 38º, 43º, 65º, and 78º, indicating that it is crystalline and has a volume [CD] of 67.83 and a S.G. of fm-3m (225). The (111), (200), (220), and (311) sequence levels for fcc AuNPs correspond to these prominent peaks. As a result, the XRD pattern clearly shows that the AuNPs produced using the microwave method with CMCTS and HA as both reductant and capping factors are crystalline in character and free of impurities due to unreacted gold ions or a polymer mixture. EDX was employed as elemental analysis tool to estimate the purity and sensitivity of the blend method of nanomaterials. However, the high purity of the scanned AuNPs sample that was analyzed by means of EDX method to confirm the presence of elements in the test samples was revealed by the EDX results, as shown in Figure 5a. As shown in the EDX sample (Fig. 5a) C, O, Na, and Au elements can be found in the graph. Because reducing and stabilizing agents (HA+CMCTS) surround the produced AuNPs, it is possible that C and O are present. Na element neglect traces are present, however. As previously noted, NaOH has been used to modify the media's pH to a strong alkaline state while also activating the reducibility capabilities of HA and CMCTS. AuNP creation, meantime, may be responsible for the occurrence of Au. The EDX graph does not include any additional or other elements that might further emphasise the purity of AuNPs as seen in the XRD figure.

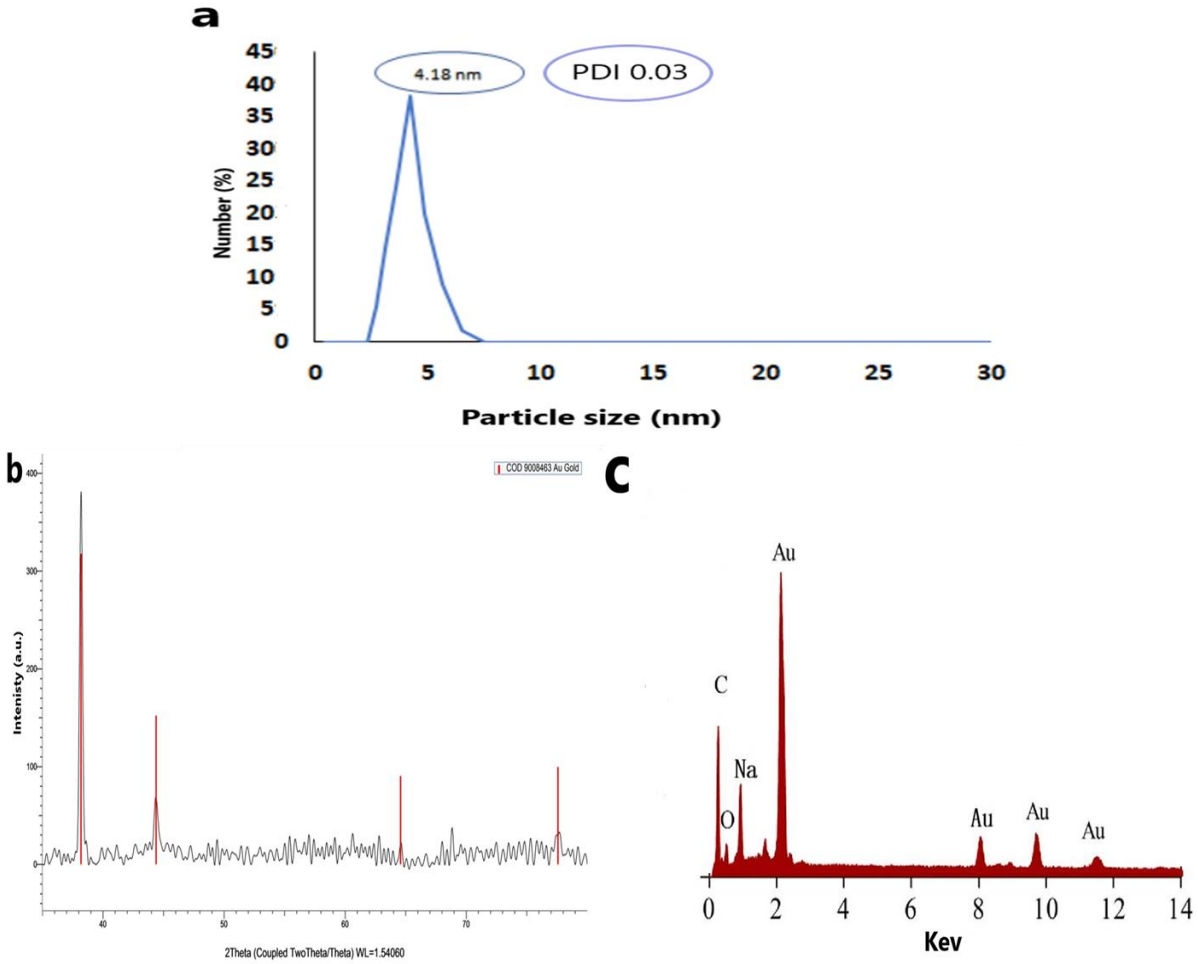

Figure 3. (a)Average particle size, (b) XRD of AuNPs and (c) EDX.

The FTIR spectrum of the HA+ CMCTS in ratios (1:3, 1:1 and 3:1 respectively) embedded gold nanoparticles showed bands at 3815, 3355, 3227, 2226, 1637, 1167 wavenumber along the spectrum. In order to identify the creation of distinctive bonds from hyaluronic acid, carboxymethyl chitosan, and bonds arising from the fraternization of these biopolymers embedded AuNPs, FTIR-ATR analysis was performed. The frequency of vibrations could indeed be contingent on the intensity of hydrogen bonds which stabilize the structure of blended polymers, and this analysis enables us to see whether functional groups are present in the CMCTS/HA crosslinked as well as their shift patterns, which might specify hydrogen interactions (1167 cm^{-1} region, C-O stretching harmonic resonance peak) [40]. The CMCTS and HA characteristic bands were seen: The amide A bond, which is close to 3355 cm^{-1}, has an NH-stretching vibration [41]. In the combined polymers, the band positions are not significantly different. As shown in Figure 4, a sharp band that originates from the carbonyl group forms at a wavelength of 1637 cm^{-1}. The projected CMCTS/HA connection is confirmed by this band. This investigation suggests that polymers surround the gold nanoparticles because they serve as surface coating molecules that keep the AuNPs from clumping together inside the particles.

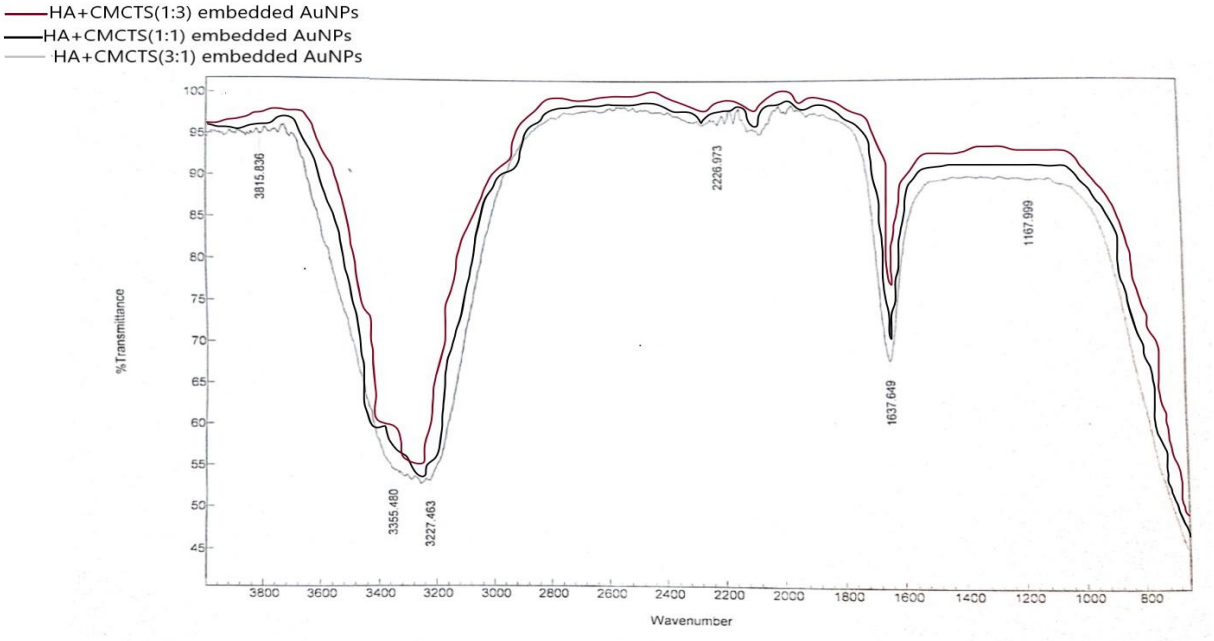

Figure 4. The FTIR spectra of caarboxymethyl chitosan/hyaluronic acid/carboxymethyl chitosan (1:3 ,1:1 and 3:1 respectively) embedded AuNPs-based materials

3.2. Antimicrobial assay for the polymers adorned AuNPs

One of the objectives of the present work was to inspect the antimicrobial influence of the established three mixtures of (CMCS+HA) polymers embedded AuNPs, combination of polymers with AuNPs were investigated for their powerful effect on *Methicillin-resistant Staphylococcus aureus (MRSA)* as Gram positive bacteria, *Klebsiella pneumonias (K. pneumonia)* as Gram negative bacteria and *Candida albicans (ATCC 10231) (C. albicans)* as example for fungi. The minimum inhibitory concentrations (MICs) of all tested groups on bacteria and fungi strain showed in table (1). Obviously, (HA + CMCTS + AuNPs) found to have anti-bacterial and fungal effect in all ratio levels comparing to positive control (the microorganism in liquid media only) with better results for combination of polymers in all ratios with AuNPs than for polymers and AuNPs separately (see Fig.5). Also, the MIC findings indicate that low concentrations of polymers and AuNPs were needed to kill the isolates of tested organisms.

Table 1 MICs (µg/ml) for all tested groups against MRSA, *K. pneumonia* and *C. albicans*

Groups	Mean MIC (µg/ml) of *MRSA*	Mean MIC(µg/ml) of *K. pneumonia*	Mean MIC(µg/ml) of *C. albicans*
CMCS+HA	41.16 ± 2.36	44.16 ± 2.36	46.16 ± 2.36
AuNPs	36.65± 2.36	32± 2.36	31± 2.36
HA+CMCTS in ratio (1:3) embedded AuNPs	24.06 ± 2.36	22±3.4	23±5.6
HA+CMCTS in ratio (1:1) embedded AuNPs	28±1.2	21±5.4	21±3.1
HA+CMCTS in ratio (3:1) embedded AuNPs	19±4.5	18±7.6	19±3.4

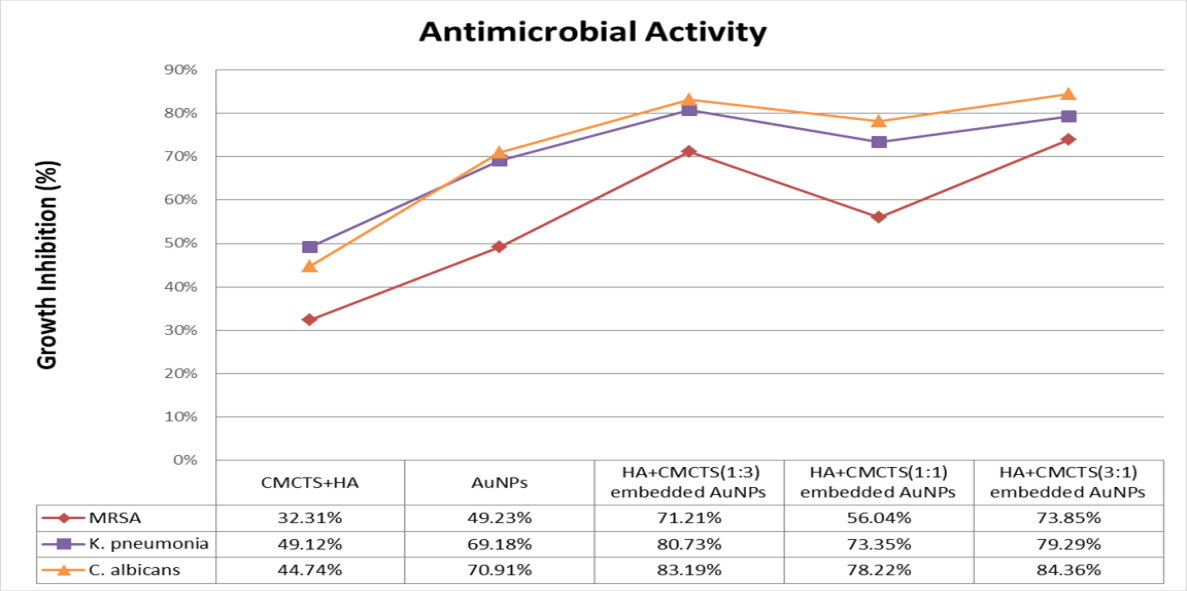

Figure 5. Graphical representation of Antimicrobial activity by growth inhibition (%) of CMCTS+HA, AuNPs and all polymer mixtures embedded AuNPs against *Methicillin-resistant Staphylococcus aureus* (*MRSA*), *Klebsiella pneumonias* (*K. pneumonia*) and *Candida albicans* (ATCC 10231) (*C. albicans*) over different concentration.

In many studies for the effect of CMCTS on both gram-positive, gram-negative bacteria and fungi. Results illustrated that polymer was effective in decreasing the MIC of all tested organisms that was in harmony with current study [42, 43]. The current work findings were in the same line with that for the study described the blending and description of HA-based hydrogels, as the results of the hydrogels were found to have anti-microbial effects [44]. The present results (Table 1 and Fig.3) are also in agreement with the Botteon, *et al* study [45] that held to Biosynthesis and characterization for gold "nanoparticles" the prepared AuNPs displayed antibacterial activity against all tested strains, demonstrating gold efficacy as an antimicrobial agent to treat infectious bacterial illnesses.

3.3. Effect of polymers adorned AuNPs on Biological parameters

Results in Figure 5 (a, b) exhibited the BW, FI values as screening for biological activity of the tested groups, after 8 weeks of HFD and ND feeding, animals of positive control group (G2) gained more body mass (BW) and showed more feed intake (FI) comparing with the negative control group (G1) that could be explain as fructose metabolism generally increases food palatability which rises feeding behaviour and as a result promote overeating. Besides, high-fructose intake encourage leptin resistance that leads to amplification of food consumption and obesity [46]. Results for BWG and FI increasing as a result for high fructose intake found in many researches [47-49] which used high fructose diet to induce obesity in rats that found to be in harmony with current work. Treated groups with natural Polymers embedded AuNPs (G5,G6 and G7) found to have lower values in BW and FI than for the positive control group, but with no significant change, these results was in line with Soliman, *et al* [49] who used AuNPs as anti-obesity in rats fed high fructose diet. Gold nanoparticles revealed a favourable profile for

potential controlling of obesity in the study of Chen, *et al* [12] that was the same with the results of current work. Also, AuNPs can work as a model to motivate medication for weight loss and avoidance for obesity-related metabolic syndromes [50]. Chitosan presented a positively effect on a number of metabolic parameters including obesity-related markers (BW, FI), which encouragement its role for the cure of metabolic disorders as obesity in many researches held on rats and human [51]. Hyaluronic acid found to have indirect role in controlling obesity by developing a modern and hopeful manner to fight obesity through a single, monthly controlled-release intra adipose dose of "Clenbuterol-modified HA thermo-sensitive hydrogel", the recently established "Clenbuterol formula" found to be effective not only in reducing body weight but also in managing the side effects of the classical oral administration of "Clenbuterol" alone [52].

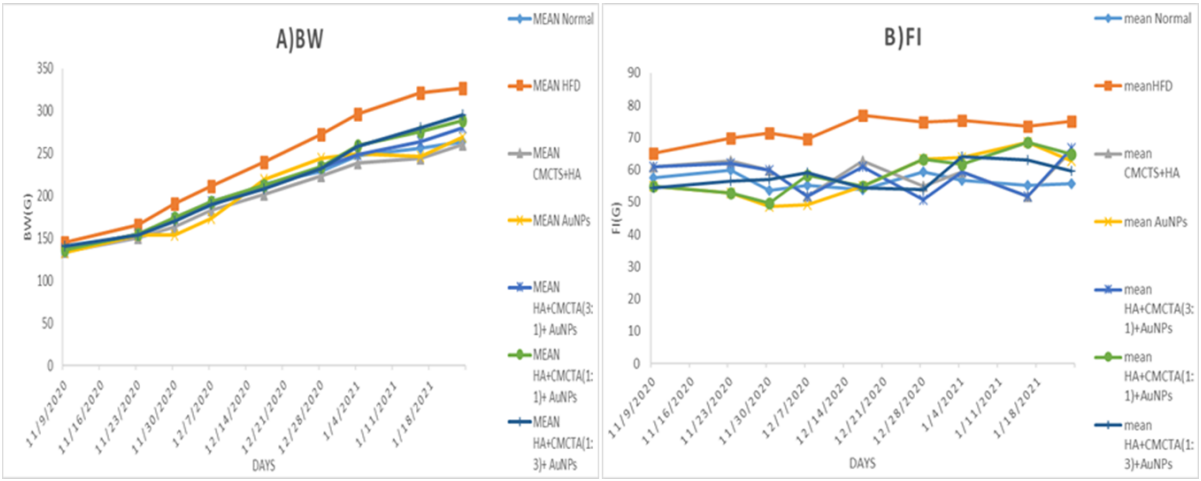

Figure 6. Effect of HA+CMCTS, AuNPs and HA+CMCTS embedded AuNPs in ratios (1:3,1:1 and 3:1 respectively) on: (a) Body Weight (BW) in gram, (b) Feed intake (FI) in gram.

3.4. Effect of polymers and AuNPs on Biochemical parameters

Obesity is becoming a main public health issue because it is linked to the progress of diseases like type 2 diabetes, cardiovascular disease, and metabolic syndromes. It was discovered that most obese people have amplified food consumption despite high circulating leptin levels, implying a state of leptin resistance, or a reduced responsiveness to leptin's appetite and weight loss suppressing effects [19].The results in Figure 7. (a, b, c, d, e, f, g and h) revealed that there was a highly significant increase ($P < 0.0001$) in TC, TG, LDL, VLDL AI, AC and CRR with a highly significant decrease ($P < 0.0001$) in HDL in rats fed the HFD diet versus the control animals. These findings found to be in harmony with the results for Soliman et al [49] whom explained that high fructose utilization causes the development of Cardiovascular diseases (CVD) by growing TC, TG, LDL, vLDL , AI,AC and CRR as well as reducing HDL in circulation. According to Newairy et al [53] the mechanism of action for HDL was that it helps scavenge extra-hepatic tissue cholesterol and the decrease in HDL concentration has led to increasing in concentrations of cholesterol and LDL levels which associated with increased risk of developing coronary heart disease (AI, AC and CRR). By using the polymers and AuNPs in the treated groups the lipid profile (TC, TG, HDL, LDL and vLDL) and atherogenic indices (AC, AI and CRR) results showed enhancement in its value comparing to the HFD group that could be explained as each used substance found to has its effect on controlling obesity and lipid profile [12, 13, 16]. As the novelty of this current work was to produce a newly hydrogel from the two polymers embedded AuNPs used not only as a drug delivery system but also in the attenuating of obesity and its complications, the results of the treated groups with polymers mixtures embedded AuNPs (G5, G6 and G7) showed reduction in TC, TG, LDL, vLDL, AI, AC and CRR levels and rise in HDL, with the best results for (G7) which had lipid profile and atherogenic indices values very close to the normal control results. These findings could be explained by the fact that Chitosan oligosaccharide compounds (CMCTS) with glucan and polyphenols groups could recover endothelial dysfunction by limiting markers of inflammation in the liver and adipose tissues and reduced serum cholesterol by promoting cholesterol accumulation in the liver, bile, and faeces via the reverse cholesterol transport pathway [14]. Furthermore, the interaction of hyaluronic acid with cell membrane receptors aids

in morphogenesis, tissue renovation, and inflammation. Ha is found in vascular plaques and is employed in angioplasty stents to prevent artery restenosis and clotting [16]. Gold nanoparticles had its effective role in reducing blood lipid levels and According to Chen et al, AuNPs therapy could be a potential novel method for controlling metabolic problems in obese people, which was consistent with our findings [54].

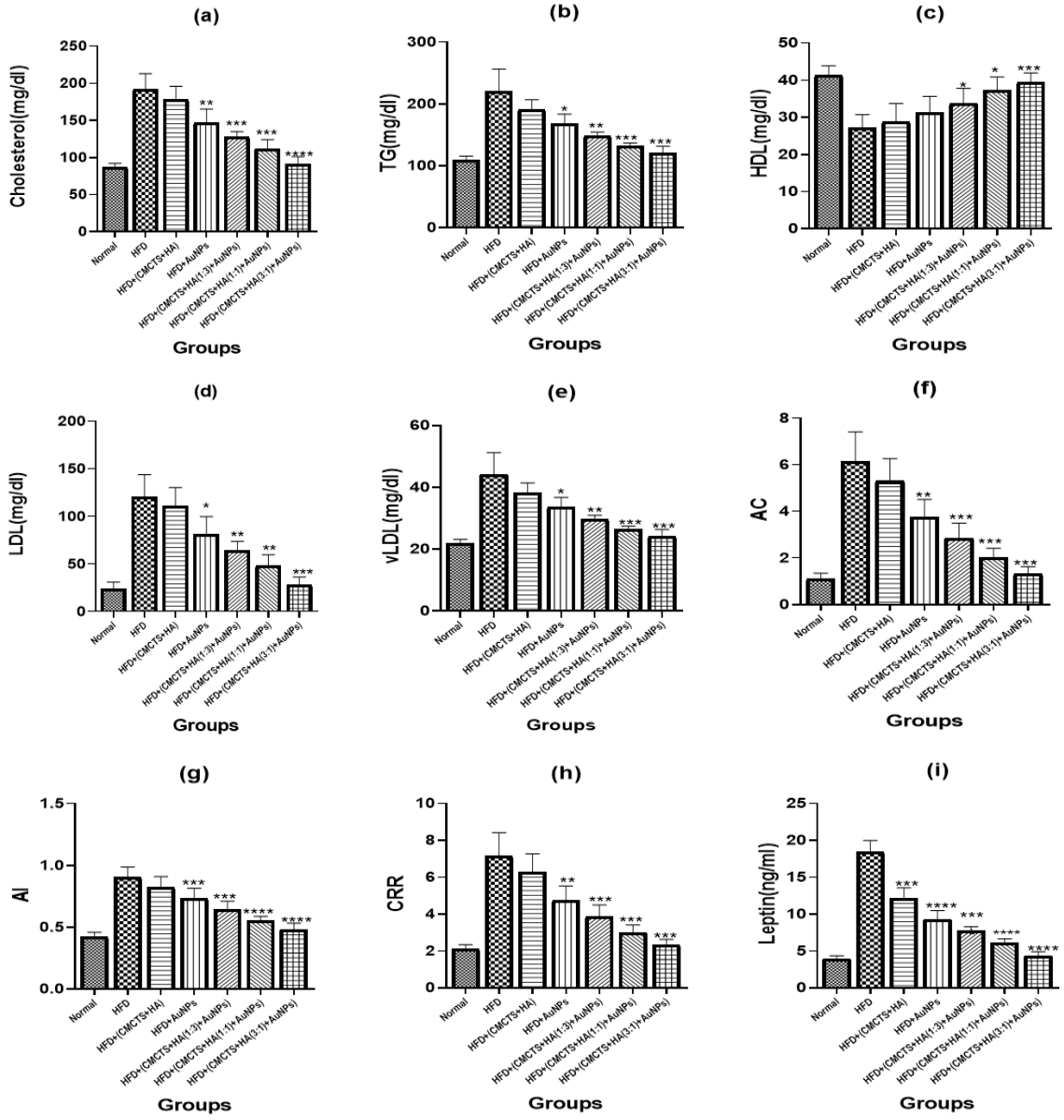

Figure 7. Effect of polymers mixture, AuNPs and polymers mixture embedded AuNPs on: (a) total cholesterol (mg/ dl), (b) serum triglycerides (mg/ dl), (c) high-density lipoprotein (mg/ dl), (d) serum low-density lipoprotein (mg/ dl), (e) very low-density lipoprotein (mg/ dl), (f) Atherogenic Coefficient (AC), (g) Atherogenic Index (AI), (h) Cardiogenic Risk Ratio (CRR) and (i) leptin (ng/ml). Where, G1 represented: Negative control group, G2: High Fructose Diet (HFD) positive control group, G3: HA+CMCTS, G4: AuNPs, G5: HA+CMCTS in ratio (3:1) embedded AuNPs, G6: HA+CMCTS in ratio (1:1) embedded AuNPs and G7 was: HA+CMCTS in ratio (1:3) embedded AuNPs. Data are expressed as mean ± SD. Statistical analysis was carried out by one-way ANOVA followed by Dunnett's multiple comparisons test. (****, ***, ** and *) Represents significant difference between HFD group and treated groups, **** ($P < 0.0001$), *** ($P < 0.0002$), ** ($P < 0.002$) and * ($P < 0.05$).

Figure 7.i showed that leptin results of investigated groups was in the same line for lipid profile results as (G2) with HFD showed 2-fold increase in leptin levels which confirm leptin resistance as a result of obesity and weight gained from HFD intake. The leptin signaling system maintains body weight using the following mechanism: To prevent overeating and increase

energy expenditure, leptin binds to leptin receptors (LEP-R) in the brain when fat cells increase. However, weight gain happens when a positive energy balance (i.e., caloric consumption exceeds energy expenditure) is maintained for crucial periods of time [17]. As mentioned for lipid profile CMCTS, HA, AuNPs showed a promising result in hyperleptinemia attenuation especially in (G7) that used CMCTS+HA in ratio (3:1) embedded AuNPs. These findings aligned with those of Bahijri et al [55], who utilized chitosan as a supplemental diet to down regulate obesity-related indicators like leptin in high-fat diet rats[55]. An oral formulation containing hyaluronic acid demonstrated significant reductions in inflammatory cytokines and leptin , indicating that it could be a safe and effective treatment for overweight patients with knee osteoarthritis [56]. The use of gold nanoparticles in methylcellulose (MC)-based hydrogels led to the development of tunable thermosensitive hydrogels for loading multimodality therapeutic ingredients to improve leptin bioactivity for obesity treatment [57].

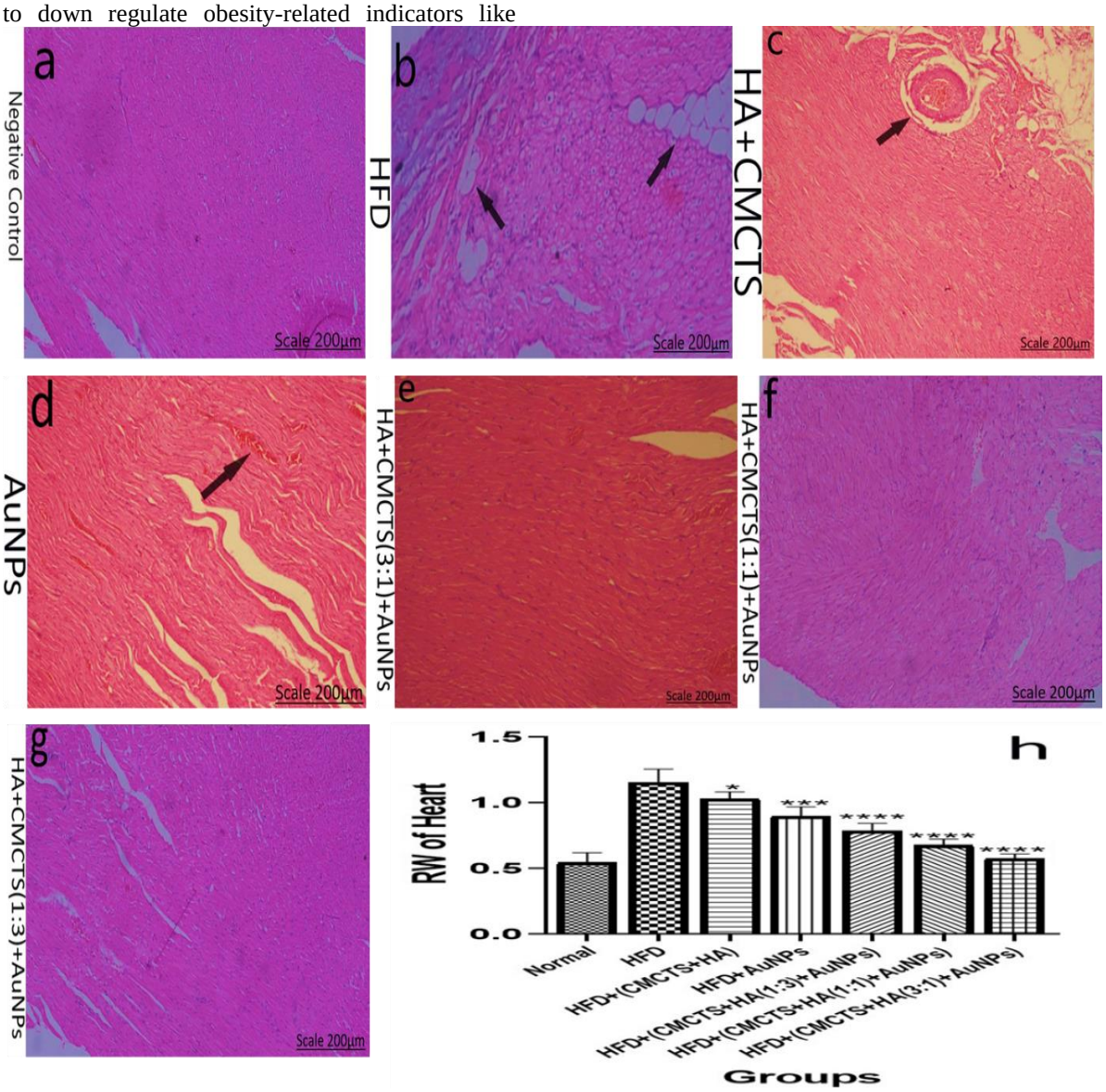

Figure 8. Photomicrograph (H&E, scale bar, 200 µm) of rats Heart for : (a) normal control rats showing normal structure , (b) HFD positive control rats' showing hydropic degeneration in cardiac muscle bundles , (c) rats received HA+ CMCTS showing congested blood vessels and perivascular edema,(d) rats received AuNPs showing congestion of blood vessels,(e) rats fed HA+ CMCTS in ratio (3:1) embedded AuNPs showing normal structure of heart muscle with few congestion of blood vessels,(f) Rats fed HA+CMCTS in ratio(1:1) embedded AuNPs showing no histopathological changes,(g) Rats fed HA+CMCTS in ratio (1:3) embedded AuNPs showing no histopathological changes and (h) relative weight of heart (RW of Heart) in different experimental groups. (****, ***, ** and *) Represents significant difference between HFD group and treated groups, **** ($P < 0.0001$), *** ($P < 0.0002$), ** ($P < 0.002$) and * ($P < 0.05$).

3.5. Histopathology and relative weight of the heart

The HFD group (G2) had the highest RW with a significantly difference values compared by the control group (see Figure 8, h). Additionally, hydropic degeneration in cardiac muscle found in Microphotograph of rat heart in Figure (8, b) that might be a result of excess consuming of fructose which represented a hazard agent for the consequence of metabolic syndrome through several mechanisms with dysfunctions in various organs and tissues as heart, these results found to be in agreement with Handayani et al [58] as they reported that dietary fat and fructose consumption promote atherosclerosis and cardiac histopathology alteration. Photomicrographs of treated groups with CMCTS+HA and AuNPs fig. 8 (c, d) congested blood vessels and perivascular edema, but nonetheless the relative weight of heart showed decrease with highly significant values (see Figure 8, h) (G3,4). Results of treated groups with HA+CMCTS in ratios (3:1, 1:1 and 1:3 respectively) embedded AuNPs revealed normal structure of heart muscle with no histopathological changes fig.8 (e, f and g) and that could be confirmed by t the heart relative wight which illustrated a very significant results with the very close values to normal control group specially in G7, that was in harmony with lipid profile and leptin results for the same group which confirms the aforementioned findings. Injecting a disulfide-cross-linked chitosan hydrogel overloaded by basic fibroblast growth factor (bFGF) into a peri infarcted zone of cardiac muscle proximately afterward Myocardial infarction (MI) in a rat model revealed that the hydrogel significantly reduced the fibrotic area of Myocardial infarction (MI), and this was additional enhanced by the bFGF-hydrogel treatment [59]. The bFGF-hydrogel had a higher synergistic effect on anti-apoptosis and pro-angiogenesis of heart cells than either bFGF or the hydrogel alone, according to immunohistochemical staining results, which matched the current work results[59].

4. Conclusion

In this present work, two types of both natural polysaccharides; hyaluronic acid and carboxymethyl chitosan are successfully mixed together in ratios (1:3; 1:1; 3:1 respectively) in order to produce 3 different blend polymers to test their effectiveness as anti-obesity agents, and each blended ratio of polymers was embedded with AuNPs by microwave technique. Then, the polymer mixtures bearing AuNPs, were successfully used in controlling lipid profile and leptin levels in albino rats induced obesity by high fructose diet (HFD) model. All treated groups (G3, G4, G5, G6 and G7) findings, displayed a noticeable decrease in lipid profile and leptin values compared with the positive control group, and disclosed also normal histopathological heart muscle structures. However, the mixture of HA/CMCTS (1:3) adorned AuNPs was more potent than the other used ratios from the polymer combination, in attenuation of hyperlipidaemia intricacy and reducing leptin resistant. Also, the antimicrobial activity for all mixtures was effective but almost the same. To conclude, both HA and CMCTS incorporated with gold nanoparticles, found to have indirect role in attenuating various biochemical and histological abnormalities resulted due to obesity metabolic disorders in experimental model of overweight rats by controlling the mechanism of action for the leptin signaling system to keep body weight and can be applied successfully in medical application as indirect diet control supplement.

5. Conflicts of interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

Abbreviations

HA: Hyaluronic Acid
CMCTS: Carboxymethyl Chitosan
AuNPs: Gold Nanoparticles
HFD: High Fructose Diet
ND: Normal Diet
TEM: Transmission Electron Microscope
SPR: Surface Plasmon Resonance
MTP: Micro Titer Plates
MHB media: Muller Hinton broth media
MIC: Minimum Inhibitory Concentration
BW: Body Weight Gains
FI: Feed Intake
H& E: Hematoxylin and Eosin stain
MRSA: *Methicillin-resistant Staphylococcus aureus*
K. *pneumonia*: *Klesiella pneumonias*
C. *albicans*: *Candida albicans*

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الملخص:

في هذا العمل ،تم استخدام مزيج حمض الهيالورونيك والشيتوزان المدمج بكمية محددة من جزيئات الذهب النانومترية للتخفيف من مستوى الدهون ومقاومة اللبتين في الفئران البدينة. حيث تم تحضير جزيئات الذهب من خلال تقنية إشعاع الميكروويف ثم توصيف خصائصها باستخدام المجهر الإلكتروني النافذ و التحليل الطيفي للأشعة فوق البنفسجية وحيود الأشعة السينيه و الأشعة السينية المشتتة للطاقة وكذلك المطيافية الفورييه لتحويل الأشعة تحت الحمراء . تم تقسيم عدد (70) من الجرذان إلى مجموعتين رئيسيتين: المجموعة الأولي (عدد 10جرذان) تغذت علي النظام الغذائي الصحي(مجموعة ضابطة سالبة) ،المجموعة الثانية (عدد 60 جرذ) تم تغذيتها علي نظام غذائي عالي الفركتوز لمدة 4 أسابيع وذلك للحث علي زيادة نسبة دهون الدم والسمنة. بعد التأكد من إصابة الجرذان بزيادة نسبة الدهون بالدم وزيادة الوزن تم تقسيم الجرذان إلى ستة مجموعات فرعية (عدد10 في كل مجموعة)،المجموعة الفرعية الأولي تغذت علي وجبة عالية الفركتوز (مجموعة ضابطة موجبة)،المجموعة الفرعية الثانية تغذت علي وجبه عالية الفركتوز مع خليط من حمض الهيالورونك/ الشيتوزان، المجموعة الفرعية الثالثة تغذت علي وجبة عالية الفركتوز مع جزيئات الذهب النانومترية، أما المجموعات الفرعية (4،5،6) تلقطت عن طريق الفم الخليط من حمض الهيالوروتيك/الشيتوزان الممزوجان بجزيئات الذهب بنسب مختلفة (1:3، 1:1، 3:1) علي التوالي، بجرعة 2 مجم / كجم من وزن الجسم / يوم لمدة 4 أسابيع . تم تقييم تأثير خليط البوليمرات والذهب منفردين و مزيج البوليمرات مع جزيئات الذهب علي البكتيريا والفطريات وقد أظهرت النتائج التأثير المضاد لنشاط البكتريا والفطريات لجميع نسب الخليط ، كانت النتائج الأفضل تأثيرا كمضاد للبكتيريا والفطريات للبوليمرات الممزوجة في جميع النسب مع جزيئات الذهب النانومترية مقارنة بالبوليمرات و جزيئات الذهب النانومترية بشكل منفصل . كما انخفضت المؤشرات الحيوية خفضت لجميع المجموعات المعالجة (3،4،5،6،7) مستويات هرمون اللبتين والدهون في الدم (الكوليسترول الكلي ، الدهون الثلاثية ، البروتين الدهني عالي الكثافة ، والبروتين الدهني منخفض الكثافة و البروتين الدهني منخفض الكثافة للغاية وكذلك مؤشرات تصلب الشرايين (مؤشر تصلب الشرايين ، معامل تصلب الشرايين ونسبة مخاطر الإصابة بأمراض القلب) مقارنتا بالمجموعة الضابطة الموجبة. علاوة على ذلك ، كان خليط حمض الهيالورونك/ الشيتوزان (1: 3) الممزوج بجزيئات الذهب النانومترية أكثر فاعلية من النسب الأخرى المستخدمة في تخفيف التشوهات البيوكيميائية والنسيجية المختلفة الناتجة عن اضطرابات التمثيل الغذائي للسمنة. علاوة على ذلك ، كشفت جميع المجموعات الفرعية المعالجة عن الفحص الباثولوجي الطبيعي لعضلات القلب .