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# Hyaluronic Acid /Carboxymethyl Chitosan Embedded Gold Nanoparticles Modulate High Fructose Diet Induced Diabetes Changes in Rat

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**Abstract**

In this current work, hyaluronic acid (HA)/carboxymethyl chitosan (CMCTs) blend, embedded with definite amount of gold nanoparticles AuNPs , are utilized to mitigate diabetic parameters in experimental model of diabetes in rats. AuNPs was produced thru Microwave radiation technique, followed by characterization using state of art analysis; UV and TEM. Afterward, 50 ”male albino rats" were divided into two main groups: Group one "n=10 rats" fed on the healthy diet (-ve control). The 2nd group fed on the high fructose diet (HFD) for 4 weeks to induce diabetes. To this end, the 2nd group, is further divided into four sub-groups. The 1st one (G2) received only HFD (+ve control), 2nd,3rd and 4th subgroups (G3, G4 and G5) received orally; mixture of both HA and CMCTS in different ratios (1:3; 1:1; 3:1 respectively) embedded all with AuNPs (0.01M), in a dose of 2 mg/kg body weight /day for 4 weeks. In addition, the effect of polymers blends with AuNPs on *Streptococcus faecalis*, *Escherichia coli* and *Aspergillus fumigatus* was also evaluated. All treated groups (G3, G4, G5) decreased diabetic biomarkers Fasting blood sugar (FBS), fasting insulin, glycosylated haemoglobin (HbA1C), "Homeostasis model assessment of insulin resistance (HOMA-IR)" index; “homeostasis model assessment of β-cell function (HOMA-β)” compared with the +ve control. Moreover, the mixture of HA/CMCTS (1:3) adorned AuNPs was more potent than the other used ratios from the polymer combination, in attenuation of diabetic intricacy, however, the antimicrobial activity for all mixture was effective but almost the same. Furthermore, all treated subgroups disclosed normal histopathological pancreatic cell structures.

***Keywords***: Hyaluronic acid, Carboxymethyl chitosan, Gold nanoparticles, Diabetes.

## 1. Introduction

One of the promising channels of research is developing biomaterials using natural polymers. Hyaluronic acid (HA) is one of the natural linear polysaccharide that formed by repeating units of D-glucoronic acid and N-acetyl-D-glucosamine disaccharide that isolated from the vitreous humor of bovine eyes in 1934 for the first time [1].

HA has a desired properties in the biomedical applications as HA consider a biodegradable, biocompatible, non-immunogenic and nontoxic polymer with a 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 characters 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].

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Chitosan with hyaluronic acid could form a new material based on such binary blends Many studies demonstrated beneficial activities of chitosan as protecting and proliferating agent for pancreatic beta cells, reducing hyperglycemia, and stopping impaired lipid metabolism associated diabetes mellitus [7]. Also, (HA-NPs) itself without any drug has therapeutic effects on inflammation of adipose tissue and resistance of insulin, that could promote insulin sensitivity and normalize glucose level in blood [8].

Gold nanoparticles used in many fields as favored materials for their unique physical and optical features[9], 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 used as a transcriber in therapeutic field due to its large surface area/volume ratio, letting their surface coated with various types of molecules including targeting and therapeutics agents[10]. One of the main reasons for using gold nanoparticles in biomedical field is that they are safe and biocompatible to both in vivo and in vitro environs [11]. Among the medicinal NPs with hypoglycemic properties, we can mention gold nanoparticles (AuNPs) and their various derivatives [12, 13].

There are numerous techniques for AuNPs synthesis such as biological, electrochemical, chemical reduction, laser, and microwave radiation besides, microwave radiation considered to be a favorable technique for the production of controlled nanoparticles without using a lot of chemicals and in no time [14], nanoparticles obtained by this technique having a great surface area and small size which enable them to infiltrate into the targeted organs [11].

Fructose commercially used as a sweetening material “fructose corn syrup” in desserts elaboration and carbonated beverages [15]. Consumption excess amount of dietary fructose may leads to adverse effects in hepatic, metabolic and vascular functions causing many diseases [16], also dietary has a high fructose revealed to cause hyperinsulinemia, hepatic-steatosis and hyperlipidemia [17].

Recently consumption of excess quantities of refined carbohydrates in beverage and food confirmed to rises the risk of dyslipidemia, insulin resistance, heart diseases and obesity[18]. An epidemiological recent study in humans found an association between sugar obtainability and diabetes prevalence[19]. Furthermore, chronic intake of Western diet characterized to be rich in sugar and saturated fat [20], proposed to play a significant role in the expansion of type 2- diabetes mellitus “T2D”, which considered as metabolic disease associated with impaired glucose, protein and lipids metabolism with hyperglycemia. This metabolic disorder is due to shortage in insulin secretion or insulin resistance action, or together that leads to several metabolic abnormalities and chronic complications, as a result of high glucose levels [21].

As for, the novelty of this current work is to study the synergistic influence of the aforementioned combination of both naturally occurring polysaccharides (HA and CMCTS) adorned AuNPs, on controlling diabetic biomarkers of albino rates induced diabetes by high fructose diet (HFD) model, through promoting the propagation and recapture of damaged β-cells which can produce insulin and consequently leads to an increase in secretory capacity of insulin.

## 2. Materials and Methods

### 2.1. Materials

Hyaluronic Acid and Carboxymethyl Chitosan were obtained from Wako Chemicals (USA, Inc.). Fructose purchased from (Safty Egypt Company Cairo, Egypt). Tetra chloroauroic acid ($HAuCl_4$), sodium hydroxide) NaOH) and chemicals for Invitro experiment were gotten from Sigma Co. (USA).

All other utilized diet ingredients such as: (Casein >85% protein, DL –methionine, choline chloride, corn starch, vitamins, minerals and other utilized materials) were purchased from Morgan Company for Chemicals, Cairo, Egypt. All used chemicals were with high purity and diet ingredients were food grade with high purity.

### 2.2. Animals

Fifty vigorous adult male albino rats "Sprague Dawley strain" weighing (150 ± 10g) purchased from (the animal colony, Helwan Farm, Vaccine and Immunity Organization, Cairo, Egypt). Rats were given time to adapt before the initiation of the experiment, rats kept at the basic conditions (temperature 22 ± 2 °C and light/ dark cycle 12:12 h) and supplied by the freshwater and the standard diet.

### 2.3. Synthesis of Gold Nanoparticles AuNPs using Microwave

Gold nanoparticles (AuNPs) was produced by microwave technique thru using Hyaluronic Acid and Carboxymethyl Chitosan mixture with three different ratios of both polymers as (1:3; 1:1; 3:1 respectively). Both HA and CMCTS were used as stabilizing and reducing agent for the formed AuNPs. In details, 0.1 g of HA and 0.1g of CMCTS was dissolved in (100 ml) of deionized water at room temperature. The pH was adjusted to 11 for each solution individually by using drops of very diluted NaOH (0.001 M), after the pH adjusting, each ratio of solution was submitted

to microwave radiation for 10 s, and 2 ml of $HAuCl_4$ (0.01 M) was added to the polymer mixtures solutions. Each mixture solution was subjected again to microwave radiation for additional 40 s, when time completed, the color was altered from colorless to deep pink color which was referred to AuNPs forming [22].

**2.4. Characterization of AuNPs**

The Surface Plasmon Resonance (SPR) of AuNPs was measured using an absorption spectroscopic approach with a dual beam UV–Vis–NIR spectrophotometer (Unicom UV 500 UV/VIS spectrophotometer) over a scanning range of (200–800 nm). High Resolution Transmission Electron Microscope (HR-TEM) (JEOL-JEM 1200) working at a high voltage of 200 kV (Tecnai G2, FEI, Netherlands) was used to image the morphology of the produced AuNPs. The average particle size of AuNPs obtained from TEM images were computed using (Image J4s) well-known software.

**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 *Streptococcus faecalis* (MTCC – 0459) (*S. faecalis*) as Gram positive bacteria, *Escherichia coli* (ATCC 25955) (*E.coli*) as Gram negative bacteria and *Aspergillus fumigatus* (*A. fumigatus*) as example for fungi, with Micro dilution Method in micro titer plates (MTP) performed to define the minimum inhibitory concentration (MIC) in which 1: 100 (v/v) of cultures of the tested strains were added to 200 µl of Muller Hinton broth media (MHB media) and dispersed in the wells of MTP with/without Compounds. The plates were then incubated with shaking at 120 rpm for 24 h at 37 °C, after the incubation period, the cell growth was read by ELIZA reader (Tecan Elx800, USA) at 620 nm. 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[23].

**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) was fed on the healthy basic normal diet (ND) only for eight weeks as a negative control group[24] .The second group (n = 40 animals) was fed HFD (fructose 50%) for four weeks to induce diabetes [25]. After confirming diabetes development, rats were divided into four subgroups (n = 10 rats/subgroup). The 1st subgroup (G2) was fed on HFD (50%) until the end of the experimental period as a positive control group. The 2nd subgroup (G3) fed HFD and orally injected with HA+CMCTS in ratio (1:3) embedded AuNPs , the 3rd subgroups (G4) fed HFD and orally injected with HA+CMCTS in ratio (1:1) embedded AuNPs and the 4th subgroups (G5) fed HFD and orally injected with HA+CMCTS in ratio (3:1) embedded AuNPs in fixed dose of 2 mg/kg body weight in aqueous solution/day for all treated groups[22]. Thirty days from launching of the treatment animals kept fasting for 8 h and weighted then anesthetized under light ether anaesthesia by inhalation; blood was withdrawn from the retro-orbital venous plexus of the eye via capillary tubes and collected in: (a) sodium fluoride containing tubes for blood glucose estimation, (b) gel activated tubes for other biochemical parameters and (c) EDTA tubes for HbA1C test ; samples was centrifuged at (4000 rpm/min) for 10 min using centrifuge [Gyrozen Low-Speed Centrifuge Model 624R Max. Speed 6000 RPM (110V, 50/60Hz)]. Plasma and serum were collected and frozen immediately at (-20°C) till analyze.

**2.6.2. Estimation of Biological Parameters**

Estimation of body weight gain (BWG) and feed intake (FI) as biological parameters was as follow: Rats weight was measured and recorded weekly then body weight gain (BWG) of rats in all groups at the end of the experiment calculated [BWG = Final weight (g) – initial weight (g)]. Daily feed intake(FI) in gram evaluated by the difference between food offered ad libitum- and feed refusals [26].

**2.6.3. Estimation of Biochemical Parameters**

Fasting serum glucose was determined by the enzymatic colorimetric method [27] , Level of fasting insulin was evaluated in serum using (enzyme-linked immunoassay) "Rat insulin ELISA kit, Glory science Co., USA" [28]. Blood hemolyzates samples were used for glycosylated haemoglobin (HbA1c) determination [29]. Homeostasis model assessment of insulin resistance (HOMA-IR) index; homeostasis model assessment of β-cell function (HOMA-β) calculated as follow: HOMA-IR = [(fasting serum insulin (µIU / l) × fasting serum glucose (mmol / l) / 22.5]; HOMA-β = [20 × fasting serum insulin (µIU/ l)] / [fasting serum glucose (mmol / l) – 3.5] [30, 31].

**2.6.4. Determination of Relative Pancreas Weight**

The animals sacrificed at the experiment end, then, pancreas carefully dissected out and weighed in grams (absolute weight). The relative pancreatic 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] [32].

**2.6.5. Histopathological examination**

Pancreas 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 [33].

**2.6.7. Statistical analysis**

Statistical analyses for data done by [Graph Pad Prism 5 software (La Jolla, CA, USA)]. The results presented as (mean ± SD) based on the number of independent investigations mentioned. One-way analysis of variance (ANOVA) was used to determine statistical significance; differences between groups were considered significant when *P-value* ($\leq 0.05$).

**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 diabetic biomarkers level of albino rats induced diabetes by high fructose diet (HFD) model, through promoting the propagation of β-cells and the rescue of damaged β-cell functions which can yield insulin and consequently leads to an increases in secretory capacity of insulin.

The production of AuNPs , was performed thru 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 [34]. 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 [35]. 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[36]. 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 $Au^{0}$ [22]. The reduction occurs as a result of the interaction of microwave-produced radiations with the precursors in the reaction scheme, resulting in the formation of reducing active species that augment and improve the reducibility effect of CMCTS and HA, obviating the need for additional reducing agents and preventing the presence of any toxins sources [37, 38].

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 determine the true wavelength in nm of the formative AuNPs. Figure 1 shows the UV–Vis of (CMCTS+HA) and polymer-stabilized AuNPs, samples of (CMCTS+HA) colloidal solution containing AuNPs were scanned to determine the absorption from 300 to 750 nm. 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.

According to published studies, AuNPs show surface Plasmon Resonance (SPR) absorption in the "520–580 nm" range [39] hence; Because of the SPR absorption of AuNPs, the aforementioned generated AuNPs in this study show a significant crisp defined absorption band at (523 nm) The presence of an SPR peak in this range, as well as the reaction mixture's synchronies colour change, supported the AuNPs blend [34, 40].

TEM images; Figure 2 (a, b and c) illustrated that, AuNPs created with small spherical size (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.

**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 *Streptococcus faecalis* (MTCC – 0459 (*S. faecalis*) as Gram positive bacteria, *Escherichia coli* (ATCC 25955) (*E. coli*) as Gram negative bacteria and *Aspergillus fumigatus* (*A. fumigatus*) as example for fungi. Results in Figure 3. (a, b and c) displayed the minimum inhibitory concentrations (MICs) of aforementioned combination of polymers + AuNPs on the tested bacterial as well as fungal strains. 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). The lowest concentrations of polymers embedded gold nanoparticles that impede the growth of the tested strains are known as the MIC.

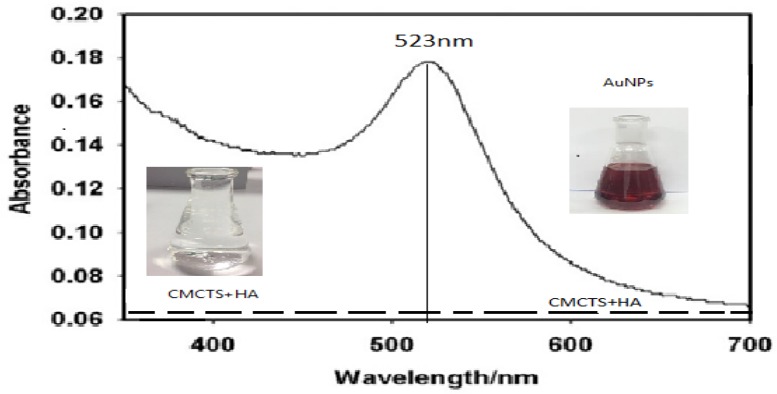


Figure 1. UV-Vis of (CMCTS+HA) Mixture and AuNPs stabilized by polymers mixture

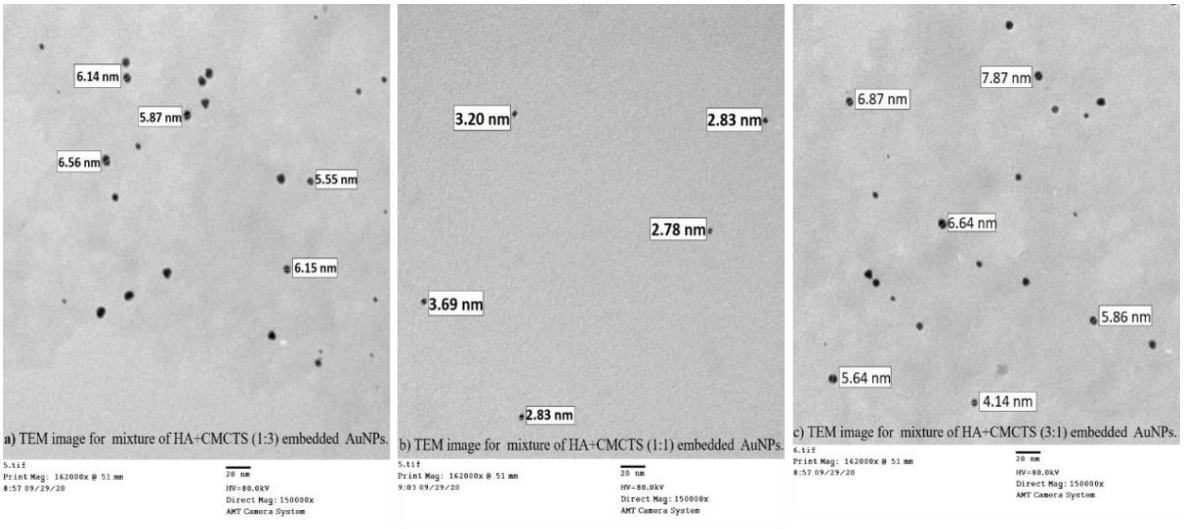


Figure 2. TEM images of all polymer mixtures embedded AuNPs, where : a) TEM image for mixture of HA+CMCTS (1:3) embedded AuNPs,b) TEM image for mixture of HA+CMCTS (1:1) embedded AuNPs and c) TEM image for mixture of HA+CMCTS (3:1) embedded AuNPs.

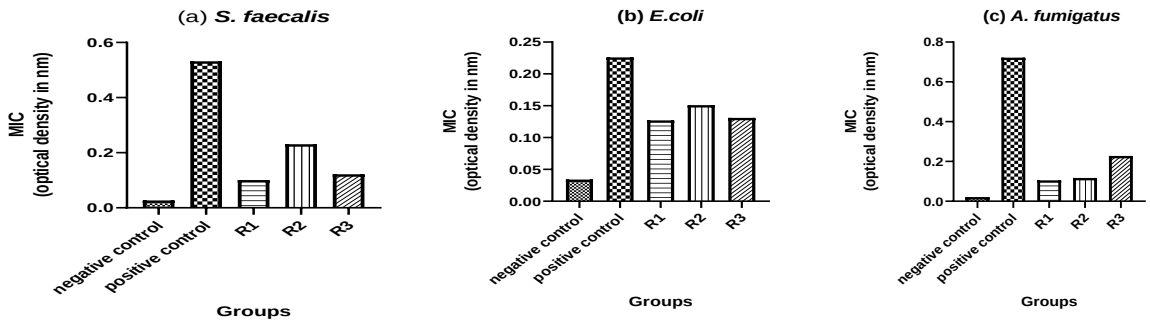


Figure 3. (a): MIC of the three formed (polymers+ AuNPs) on *Streptococcus faecalis* (MTCC – 0459) (*S. faecalis*) as Gram positive bacteria. (b) MIC of the three formed (polymers+ AuNPs) on *Escherichia coli* (ATCC 25955) (*E. coli*) as Gram negative bacteria. (c) MIC of the three formed (polymers+ AuNPs) on *Aspergillus fumigatus* (*A. fumigatus*) as example for fungi. Where, R1 represented : HA+CMCTS (1:3) embedded AuNPs, R2: HA+CMCTS(1:1) embedded AuNPs and R3 was: HA+CMCTS(3:1) embedded AuNPs.

In a study for the effect of CMCTS on both gram-positive, gram-negative bacteria and fungi illustrated that polymer was effective in decreasing the MIC of all tested organisms [41], that was in harmony with this study. In a study described the blending and description of HA-based hydrogels, according to the findings The hydrogels were found to have anti-microbial effects, as found in that work [42]. In agreement with the Botteon, et al. [43] study, that held to Biosynthesis and characterize for gold nanoparticles the prepared AuNPs displayed antibacterial activity against all tested strains, demonstrating gold efficacy as an antimicrobial agent to treat infectious illnesses.

**3.3. Effect of polymers adorned AuNPs on Biological parameters**

Results in Figure 4 (a, b) exhibited the BWG, 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 (BWG) and showed more feed intake (FI) comparing with the negative control group (G1), that could be explain as Fructose metabolism generally increases food palatability that may rise feeding behaviour and as a result promote overeating. Besides, high-fructose intake may encourage leptin resistance, which may lead to amplification of food consumption and obesity[44].

Results for BWG and FI increasing as a result for high fructose intake found in many researches[45-47] , who used high fructose diet to induce diabetes in rats that led to increase in body weight gain and feed intake, that found to be in harmony with current work. Treated groups with natural Polymers embedded AuNPs (G3,G4 and G5) found to have lower values in BWG and FI than for the positive control group, but with no significant change, these results was in line with Soliman, et al. [47] , 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. [48], that was the same with results of current work. AuNPs can work as a model to motivate medication for weight loss and avoidance for obesity-related metabolic disorders [49]. Chitosan presented a positively effect on a number of metabolic parameters including obesity-related markers (BWG, FI), which encouragement its role for the cure of metabolic disorders as obesity in many researches held on rats and human [50]. 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 side effects of the classical oral administration of "Clenbuterol" alone [51].

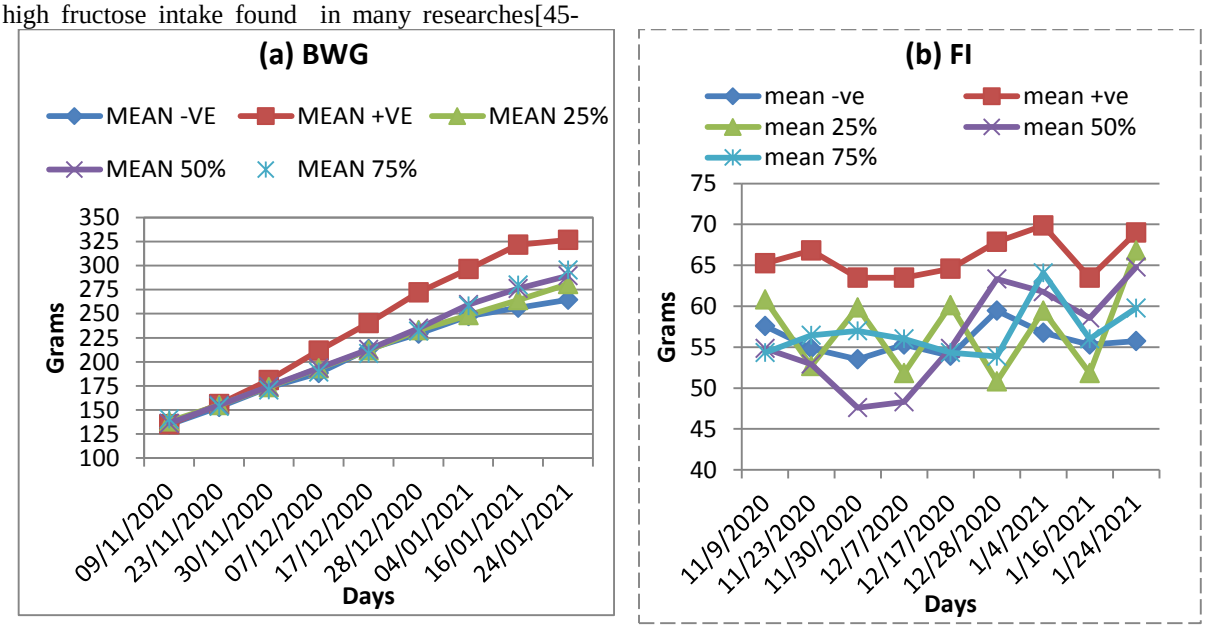


Figure 4. Effect of polymers (CMCTS, HA embedded AuNPs) on: (a) Body Weight Gain (BW) in gram, (b) Feed intake (FI) in gram, Where, G1 represented negative control group, G2: High Fructose Diet (HFD) as positive control group, G3: HA+CMCTS in ratio (1:3) embedded AuNPs, G4: HA+CMCTS in ratio (1:1) embedded AuNPs and G5 was: HA+CMCTS in ratio (3:1) embedded AuNPs.

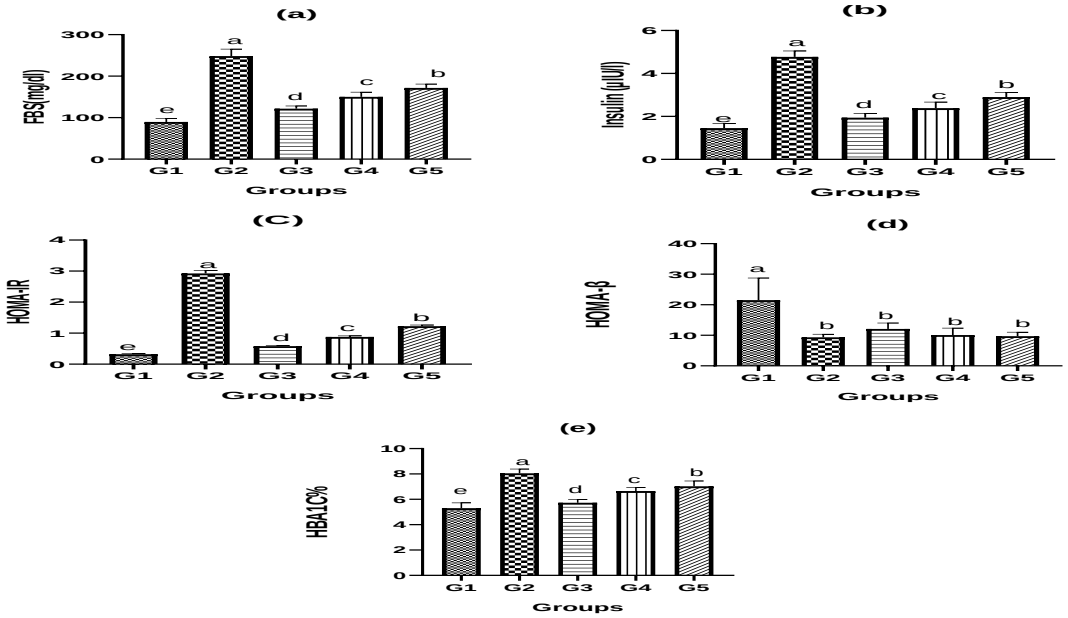


Figure 5. Effect of polymers (CMCTS, HA embedded AuNPs) on: (a) fasting blood glucose (mg/ dl), (b) serum fasting insulin (µIU/ ml), (c) HOMA-IR, (d) HOMA- β and (e) HBA1C% value in different experimental groups. Where, G1 represented: Negative control group, G2: High Fructose Diet (HFD) positive control group, G3: HA+CMCTS in ratio (1:3) embedded AuNPs, G4: HA+CMCTS in ratio (1:1) embedded AuNPs and G5 was: HA+CMCTS in ratio (3:1) embedded AuNPs.

$^{(a-e)}$ Represents the mean value ± SD. (n=10 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. ($P < 0.05$)

**3.4. Effect of polymers and AuNPs on diabetic parameters** Results in Figure 5. (a, b, c, d and e) revealed that Fasting blood glucose, Fasting Insulin, HbA1C and HOMA-IR of positive control group (G2; HFD) were significantly higher than that of the healthy control group (G1), while HOMA-β in (Fig. 4d) showed low level for all treated groups than the control group, HOMA-β found results explained by Abdel-Rahman et al. [52] with development of insulin resistance because of chronic utilization of HFD or the dropdown of β-cell function as it becomes incapacitate to yield a suitable amount of insulin. The investigated treatments in G3, G 4 and G 5 gave acceptable influence on diabetic parameters (FBS, Insulin, HbA1C, HOMA-IR and HOMA-β) with a highly significant values ($P < 0.0001$) that was in agreement with several researches used chitosan as antidiabetic substance [53-55]. Also for the same purpose, AuNPs used in diabetic parameters limitation in many studies[12, 56-58].

An empty HA-NPs have not any medication, found to have a healing effect on adipose tissue inflammation, upgraded insulin sensitivity and resistance, and controlled blood glucose levels [8]. In general G3 (with HA +CMCTS ratio (1:3) embedded AuNPs) in (2 mg/kg body weight/day) dose found to has the best control on all tested diabetic parameters (FBS, Insulin, HbA1c, HOMA-IR and HOMA-β) results with results more or less near to these of the control group, explanation for that G3 was the most effective group attributed to the ratio of polymers used that containing higher amount of CMCTS than HA, which in turn, several studies have verified the useful actions of it in reducing hyperglycemia, pancreatic beta cells proliferating and protecting, and avoiding impaired lipid metabolism related to diabetes disease. Additionally, it has been utilized in formulating multiple styles of micro/nano-carriers for the transfer of different antidiabetic medications, as insulin [7].

**3.5. Histopathology and relative weight of the Pancreas**

The HFD group (G2) had the highest RW with significantly difference compared by the control group (see Figure 6). Additionally, diffuse necrosis and fatty degeneration found in Microphotograph of rat Pancreas in Figure (7,b) might be a result of excess consuming of fructose which represented a hazard agent for the epidemic of metabolic syndrome (MetS) , with dysfunctions in various organs and tissues containing islet and β-cells of pancreas, dysfunction due to excess fructose intake has additional side effects as lipotoxicity and increasing systemic resistance of insulin [59]. Result of this current work, found to be lined with findings of Pokrywczynska, et al [60] in a study

aimed to assess the influence of fructose-rich diet on the in vitro function of pancreatic islets which resulted in increasing size and number of Pancreas islet in fructose-fed rats associated with tissue dysfunction and damaged morphology of cells. Groups (3, 4) that fed on HFD and received (HA+ CMCTS) in ratios of (1:3 and 1:1 respectively) embedded AuNPs, had a no significant change in relative weight of Pancreas (Figure 6). However, the change in results of RW was slight. Microphotograph of rat Pancreas in Figure 7(c, d) showing pancreatic normal structure of cells, that suggested to be related of the presence of CMCTS with high ratio. These data were in coherence with Akande and Fasheun [61], that results which pertain the histological investigation of the pancreas of diabetic rats received two doses of chitosan revealed amplified islet cells group paralleled with the diabetic group. Finally, G5 (HA +CMCTS (3:1) with AuNPs also found to have a slightly low level of RW (see figure 6) and histopathological results of normal structure but with mild congestion of blood vessels (Figure 7e). In agreement with the current study Selim et al.[62] showed the improving role of AuNPs on antioxidant in Wistar diabetic male rats with autism spectrum disorder (ASD), that beta cells shown euchromatic nuclei with no indication of separation of nuclear membrane which might clarify the renewing ability of AuNPs.

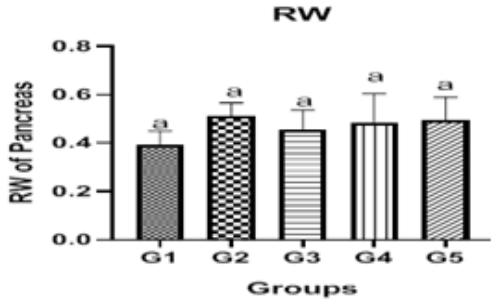


Figure 6. Effect of polymers (CMCTS, HA embedded AuNPs) on relative weight of Pancreas (RW Pancreas) in different experimental groups, Where, G1 represented: Negative control group, G2: High Fructose Diet (HFD) as positive control group, G3: HA+CMCTS in ratio (1:3) embedded AuNPs, G4: HA+CMCTS in ratio (1:1) embedded AuNPs and G5 was: HA+CMCTS in ratio (3:1) embedded with AuNPs.

$^{(a-e)}$ Represents the mean value ± SD. (n=10 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. ($P < 0.05$)

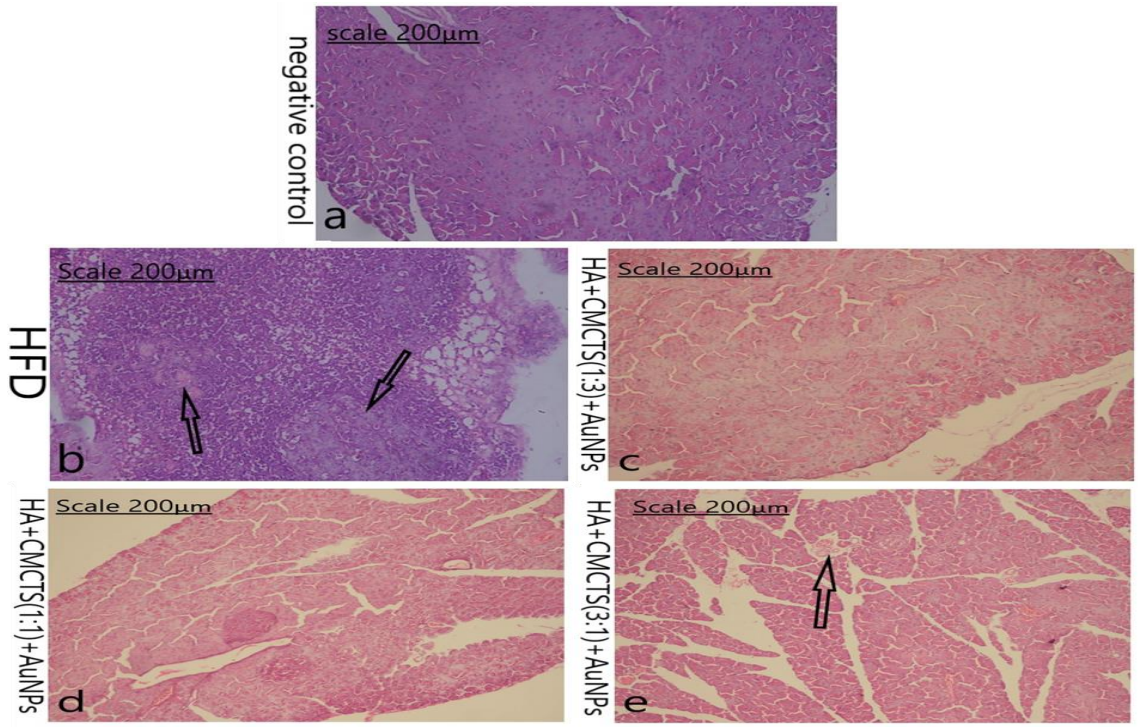


Figure 7. TS Microphotograph of rats Pancreas for : (a) normal control rats showing normal structure (HE, x200), (b) HFD positive control rats' showing diffuse necrosis and fatty degeneration (HE, x200), (c) Rats fed HA+ CMCTS in ratio (1:3) embedded AuNPs showing pancreatic normal structure of cells (HE, x200),(d) Rats fed HA+CMCTS in ratio(1:1) embedded AuNPs showing normal structure (HE, x200) and (e) Rats fed HA+CMCTS in ratio (3:1) embedded AuNPs showing normal structure with mild congestion of blood vessels (HE, x200)

## 5. 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-diabetic 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 diabetic biomarkers level of albino rats induced diabetes by high fructose diet (HFD) model. All treated groups (G3, G4, G5) findings, displayed a noticeable decrease in diabetic biomarkers fasting blood sugar (FBS), fasting insulin, glycosylated haemoglobin (HbA1C), homeostasis model assessment of insulin resistance (HOMA-IR) index; homeostasis model assessment of β-cell function (HOMA-β) compared with the positive control group, and disclosed also normal histopathological pancreatic cell 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 diabetic intricacy. 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 diabetes parameters, in experimental model of diabetic rats, and can be applied successfully in medical application as indirect diet control supplement.

## 6. Conflicts of interest

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

**Abbreviations**

| | |
|---|---|
| AuNPs : Gold Nanoparticles | HOMA-IR : Homeostasis Model Assessment of insulin resistance index |
| TEM : Transmission Electron Microscope | HOMA-β : Homeostasis Model Assessment of β-cell function |
| HFD : High Fructose Diet | BWG : Body Weight Gains |
| ND : Normal Diet | FI : Feed Intake |
| HA : Hyaluronic Acid | MIC : Minimum Inhibitory Concentration |
| CMC : Carboxymethyl Chitosan | *E. coli : Escherichia coli* |
| *S. faecalis : Streptococcus faecalis* | *A.fungus : Aspergillus fumigatus* |
| H& E : Hematoxylin and Eosin stain | MTP: Micro Titer Plates |
| MHB media: Muller Hinton broth media | SPR : Surface Plasmon Resonance |

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