The Synthesis and Characterization of Gold Nanoparticles with Polyunsaturated Oils Contribute to Hypolipidemic and Anti-Obesity Activities in Vivo

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Abstract
This study aimed to evaluate the hypolipidemic, and anti-obesity effects of polyunsaturated oils, mixed with gold nanoparticles (AuNPs). AuNPs were prepared by the oleic acid pyrolysis method. The sample was characterized by UV spectroscopy and transmission electron microscope (TEM). Sixty adult male rats were divided into 2 main groups. Group one (n=10 rats) was fed on the healthy diet (serves as basic control). The other group was fed on the high fructose diet (HFD) for four weeks and then was divided into five subgroups. 1st subgroup was fed on HFD only, 2nd and 4th subgroups were treated with n-3 PUFAs oils while 3rd and 5th treated with n-3 PUFAs oils mixed with AuNPs. Treatment of obese rats with n-3 PUFAs mixing with AuNPs revealed a significant decrease in in weight gain accompanied by an improvement of lipid profile. Histological examination of the heart indicated a marked improvement in the architecture and marked decrease of relative weight of heart in treated rats. It may be concluded that mixing of PUFAs oils with AuNPs could have the ability to lower weight gain of obese rats and relieved the various biochemical and histological abnormalities resulted due to obesity metabolic disorders.

Keywords: obesity; hyperlipidemia; polyunsaturated oils; nanoparticles

Introduction
Gold nanoparticles (AuNPs) are now well known for their valuable properties of biocompatibility, low cytotoxicity and cell regulatory effects that can be used for medical prophylactic and therapeutic purposes [1]. Chen [2] provided significant proof that AuNPs could be used as a proposed therapeutic agent in the therapy and suppression of obesity and lipid disturbances. However, AuNPs do not have negative effect on the human body and have a good safe guarantee in the human edible field [3].

ALA (α-linolenic acid), DHA (Docosahexaenoic acid), EPA (Eicosapentaenoic acid), are vital n-3 polyunsaturated fatty acids (n-3PUFAs) found in fish and certain vegetables (as flaxseeds). n-3 PUFAs regulated numerous cellular functions, signaling pathways and gene expressions as they influence the fluidity of phospholipid bilayers and the function of membrane proteins [4]. Additionally, n-3 PUFAs have hypo-triglyceridemic, anti-obesity properties and are effective against metabolic syndrome in humans [5].

Flaxseed is a plant source of n-3PUFAs as practical food, constituted 36% to 40% flaxseed oil (FLO). ALA is its main component (57%) [6] Which is the precursor of DHA and EPA synthesis [7]. Maree et al [8] concluded that daily intake of fish oil (FIO) was the foremost economical n-3PUFAs in decreasing TC and LDL and in elevation of HDL level. So, it can protect against coronary heart
disease (CHD) and the hepatic parenchyma also. The American Heart Association recently advocated the supplementation of n-3PUFAs (EPA + DHA) to patients with widespread CHD or in heart failure with lower left ventricular ejection fraction to alleviate mortality [9].

In general, the occurrence of metabolic disorders, obesity, is a significant risk factor [10]. Obesity, described as the excessive accumulation of fat in the body that damaged health, is a chronic disease with accelerated spread [11]. Approximately 60-70% of fatty (obese) patients have dyslipidaemia that include high blood serum TG (triglyceride), VLDL (very low-density lipoproteins), Apo B (apolipoprotein B), total cholesterol (TC) and low blood serum HDL levels (high-density lipoproteins) as Bays et al [12] demonstrated.

Large amounts of fructose lead to increased lipid ApoB100, LDL levels [13, 14] known as an atherogenic lipid profile [15]. So, fructose added to induce obesity and its associated dyslipidemia. The current study aimed to evaluate the hypolipidemic, and anti-obesity effects of polyunsaturated oils (FLO, FIO), mixed with AuNPs on rats receiving high fructose diet.

**Experimental Section**

**Instruments**

TEM (JEOL-JEM 1200) was used to measure the AuNPs images. The TEM was working at a voltage equal to 90 kV. For the TEM estimations, a drop of the sample containing AuNPs was put on a copper grating enveloped with indefinite carbon. After enabling the film to stand for two minutes, the extra solution was expelled using a drying paper, and the grid was permitted to dry before the examination.

**Materials and Chemical Reagents**

Flaxseed oil (FLO) and Fish oil (FIO) were purchased from Everline Company, 6-October City, Giza, Egypt. Fructose was got from El-Gomhouria Company for chemicals and drugs, Cairo, Egypt. Tetrachloroauric (HAuCl₄) and oleic acid were got from Sigma-Aldrich (Cairo, Egypt).

**Preparation of AuNPs**

AuNPs were prepared by oleic acid pyrolysis method according to [16-18] and modified by Al-Sherbini et al [19].

**Animals**

Sixty healthy adult male albino rats, weighing about 130 ± 10 g were used in the current experiment. All animals were housed individually in cages in a well-ventilated room at the Animal House of Nutritional chemistry and metabolism department, National Nutrition Institute (NNI) - Healthy Minster, Cairo, Egypt. The animals were kept below the basic conditions (12:12 h light: dark cycle and 22 ± 2°C temperature). They were fed on the standard diet and freshwater ad libitum and supplied. Maintenance and care of the experimental animals were in conformity with the International Guiding Principles for Animal Research.

**Experimental design**

Rats were fed on the standard diet for a week for adaptation then, divided into two main groups. The first group (G1) (n=10 rats) was fed only on the standard diet according to Reeves et al [20] for eight weeks, considered as a healthy control group. The second group (n= 50 animals) was fed on the high fructose diet (HFD; fructose 50%) for four weeks, according to Rajaskar et al [21]. Then divided into 5 subgroups (n=10 rats/subgroup).  The first subgroup (G2) continued fed on HFD (50%), considered as the unhealthy control group. The second and fourth subgroups (G3& G5) were fed on HFD+FLO (10%) and HFD+FIO (10%) respectively.  The third subgroup (G4) and the fifth subgroup (G6) were fed on HFD (50%) +FLO/FIO (10%) mixed with 17 ppm AuNPs according to Al-Sherbini et al [19].

**Methods**

**Biological evaluation**

Calculated feed intake (FI), body weight gains (BWG) and feed efficiency ratio (FER) indicators were used for the biological assessment of offered diets where:

Daily feed intake (FI; in grams) was calculated by subtracting the amount of food remaining in the cage from the amount of food served to each animal daily [22].

Changes in body weights (BWG) of rats in all groups were recorded weekly throughout the experimental period and weight gain was calculated for every group at the end of the feeding period (8 weeks). BWG = Final weight (g) – initial weight (g).

Feed efficiency ratio (FER) = BWG (g/day) / FI (g/day) [23].

**Biochemical assays**

Blood samples were collected into plain tubes. All samples were centrifuged at 4000 rpm for 10 min at 37 °C, and the serum was separated and stored at −20 °C until analysis. Serum TC and HDL were determined according to Burtis et al [24], serum TG was assessed according to [25], the serum concentration VLDL was calculated according to the following formula: (VLDL concentration = Serum TG / 5) and the serum LDL concentration was calculated according to the following formula: LDL = TC—(HDL + VLDL) [26].

Calculation of Atherogenic index (risk ratio 1) according to the formula of Wilson et al [27]:

\[ \text{Atherogenic index (AI)} = \frac{\text{TC}}{\text{HDL}}. \]

**Determination of Relative Heart Weight:**

At the end of experiment hearts were weighted and their ratios/body weight was calculated. The following equation calculated the comparative weight (RW) of the heart [28].

\[ \text{RW} = \left( \frac{\text{heart weight}}{\text{final body weight}} \right) \times 100. \]

**Histopathological examination**

Animals were instantly dissected to obtain the heart from each animal and rinsed with a saline isotonic solution (0.9% NaCl) to remove the excess of blood, cleaned, fixated at 10% formalin for 1 day, dehydrated, cleared, and then embedded in paraffin wax. Paraffin blocks were split into four-micron dense parts, and then stained for regular histopathological research with hematoxylin and eosin (H & E) [29].

**Statistical analysis**

Data were presented as mean ± SE (standard error). Variance analysis (ANOVA) was done followed by the post-hoc least significant difference test (LSD) to test the research hypothesis. Data analyses were performed using the Science Statistical Package (SPSS) version. A two tailed P value of < 0.05 was considered statistically significant [30].

**Results and discussion**

It is well established that AuNPs' optical absorption spectrum originated from the surface Plasmon resonance (SPR) and 30-50 nm nanoparticles showed a sharp band in the 520-530 nm region [31]. Fig. 1a, b shows the prepared sample's absorption spectrum and TEM. Fig. 1a indicated that there are two bands of Plasmon absorption of AuNPs. The first is a wideband centered around 542 nm with a visible shoulder at 576 nm and the second at 668 nm. The observed spectra may be due to the non-spherical shape of the nanoparticles. Fig. 1b showed the TEM morphological shape with the average sizes is about 40 nm ± 10 and most of the nanoparticles are in prisms shape.

**Effect of Polyunsaturated oils (FLO / FIO) and AuNPs on obesity parameters**

Obesity is a degree of excess weight that represented as a risk factor for developing different diseases [32] including metabolic syndromes and diabetes [33] through an inflammatory mechanism [34].

The results in Fig. 2a, b showed that the FI and BWG values of the positive control group were significantly increased compared with the negative control group (G1). This may be well attributed to one of the sweetest sugar (fructose), sweetness usually improves the palatability of food. Encouraged palatability may increase feeding behavior and thus lead to overeating [35]. Furthermore, by strengthening dopaminergic pathways, fructose and sucrose can enhance tastefulness and initiate addictive behaviors such as binging and, part dependence [35, 36]. Nutritional fructose diminishes excursions of leptin relative to isocaloric nutritional glucose. Fructose is less powerful than glucose in smothering the orexigenic hormone ghrelin [35]. Pereira and colleagues [37] observed that elevated consumption of fructose may contribute to the obesity epidemic and metabolic complications as it impacts the central nervous system and may disturb the control of hunger and satiety.

Polyunsaturated oils (FLO/FIO) treated groups had highly significant reduction in FI and BWG; HFD+FLO, HFD+FIO (P < 0.0001) (as shown in Fig. 2 a, b). Albracht-Schulte et al [38] proposed that n-3 PUFA's may improve the body’s structure and counteract metabolic changes associated with obesity that modulate lipid metabolism. They also could manage adipokines including adiponectin and leptin.

The treated groups with AuNPs (G4 & G6) had the lowest BWG and FI. Moreover, HFD therapy with FIO + AuNPs does not lead to significant increase in FI and BWG as a contrast to the negative control group as shown in Fig.2 a, b. These results were in line with those of Jane et al [39] who demonstrated that AuNPs lowered mice’s fat mass and metabolic diseases when fed a high-fat diet. The treated groups with FIO had lower FI and BWG than that of the groups treated with FLO as in Fig. 2 a, b. This might be due to FIO's bioactive compounds that
could help to raise the fat oxidation rate, diminish cholesterol, and increase satiation. These are vital together to decrease the general adipose tissue in the body and to restore the body to a healthy weight as Kundam et al [40] reported. AuNPs could work as a fresh paradigm for weight loss therapies and interference with metabolic disorders related to obesity and as a useful tool for evaluating biological mechanisms [10].

**Effect of n-3 PUFAs oils (FLO / FIO) and AuNPs on lipid profile analyses**

HDL helps scavenge extra-hepatic tissue cholesterol and decrease the concentration of HDL has led to increased concentrations of cholesterol. There is evidence that increased serum cholesterol and LDL levels are associated with increased risk of developing CHD [41].

From Fig. 3a, b, c, d, e, f there was a highly significant increase (P < 0.0001) in AI, LDL, TG, VLDL, and TC and a highly significant decrease (P < 0.0001) in HDL was observed in rats fed the HFD diet versus the control animals. Zhang et al [42] explained that high fructose utilization causes the development of CVD by growing VLDL, TG, TC, LDL, as well as reducing HDL in circulation. HFD induced overproduction and secretion of VLDL, the early signs of cardiovascular metabolic diseases [43], where the induction of hepatic de novo lipogenesis by activation of Sterol regulatory element-binding proteins-1c (SREBP-1c) plays an important function [44]. At the same time, HFD directly affects plasma LDL by diminishing the expression of hepatic LDL receptors [45]. Yoo and others [46] concluded that HFD, as well as high-fat diet, had adverse effects on CVD-related parameters such as artery wall thickness, serum TG, and total fat weight in growing rats as it is mainly converted into liver fat and then directly secreted into the blood as VLDL [47].

The group treated with FLO (10%) showed a highly significant reduction in TG, LDL, VLDL, AI (P < 0.0001) and TC (P < 0.01), but a significant increase in HDL (P < 0.05) compared to the HFD group. Such findings were in harmony with Akrami et al [48]; Hodson and colleagues [49] indicated that regular consumption of FLO could be a preventive strategy for metabolic syndrome (i.e. decreased rates of LDL, TG, TC) and a mechanism of treatment for high-risk individuals. This might be due to the antioxidant activity of FLO reducing oxidation of LDL, while the elevation of oxidized LDL plays a role in atherogenesis development [50].

The FIO treatment not only seemed to alleviate the increase induced by HFD on TC, TG, LDL, VLDL, and AI but also improved HDL concentration. n-3 PUFAs are abundant in marine fish, namely DHA and EPA, and function as a natural anti-inflammatory and hypolipidemic agents enhance different elements of metabolic syndrome [51, 52]. Daily intake of FIO was the foremost effective PUFAs in decreasing TC and LDL and increasing good cholesterol (HDL) levels. It is, therefore, defending against CHD and atherosclerosis as Maree et al [53] terminated. Tappy [54] noted that some metabolic disorders like chronic inflammation, high blood pressure, and dyslipidemia could be improved by the supplementation of n-3 PUFAs like EPA & DHA. In strong agreement with Song et al.'s study [55] DHA & EPA significantly improved serum HDL with a corresponding decrease in AI (a biomarker for atherosclerosis).

The groups treated with AuNPs and PUFAs oils (FLO/ FIO) had the lowest TC, TG, LDL, VLDL and AI while had the highest HDL when compared with all HFD groups (G2: G6) as shown in Fig. 3a, b, c, d, e, f. These findings were in good agreement with the results of Patil et al [56] who noted that AuNPs had lipid-lowering effects, HDL levels were significantly elevated, suggesting a reversed atherogenic risk that could result in reduced phospholipid cholesterol acyltransferase activity, which successively contributes to blood serum lipid regulation. Results of Chen et al [10] recommend a reduction of the AuNP's lipid effect and long-run safety and profit to the liver. AuNPs-treated mice were protected against the event of HFD-induced glucose intolerance likewise as hyperlipidemia.

**Histopathology and relative weight of the heart**

The HFD group had the highest RW and was significantly different from the control group (see Fig. 4). Additionally, intermyocardial oedema dispersed the muscle fibers far away from each other associated with inflammatory cells infiltration observed in the control group HFD (Fig. 5b).

From Fig. 4, It was evident that the RW heart for n-3 PUFA oils (FLO / FIO) decreased significantly compared to the positive control group (G2) (P < 0.0001). Rat’s heart from G3 (HFD + FLO) revealed intermyocardial oedema and few infiltrations of.
intramuscular inflammatory cells (Fig. 5c); whereas the heart of groups fed with HFD-FIO displayed no histological changes (Fig. 5d).

RW Heart of HFD-FLO-AuNPs, HFD-FIO-AuNPs groups were 0.29, 0.27 in comparison with 0.47 of HFD control group (illustrated in Fig. 4). Rats fed HFD + FLO or FIO + AuNPs showed no histopathological changes (Fig. 5 e, f). Also, the studies had been set up by Han et al [57] suggested that improvement effect of FLO on atherosclerosis & lipid profiles may be associated with ACC (Acetyl-CoA carboxylase), SREBP-1c (sterol regulatory element-binding protein-1) and SREBP-2 regulation. Previous studies to understand the cytotoxicity of AuNPs showed that AuNPs did not show any toxicity compared to gold ions [58]. "Parveen et al[59 ] confirmed that" AuNP administration showed no toxicity in the day-to-day activity of male and female rats. Besides, the continuous administration of AuNPs intra-articular has no toxic effects on the internal organs (lungs, kidneys, spleen, and liver) [60].
Fig. 2: Effect of polyunsaturated oils (FLO and FIO) and AuNPs on a) feed intake, b) body weight gain (BWG), c) feed efficiency ratio (FER) value in different experimental groups.

- Represents the mean value ± S.E. (n=10 rats / group). Means that do not share a letter are significantly different using One-way ANOVA. (P < 0.05)

- Represents significant difference between control group and treated group using student's unpaired t-test, ¥ (P< 0.0001), and € (P< 0.05).

- Represents significant difference between HFD group and treated group using student's unpaired t-test, a (P< 0.0001) and ** (P < 0.01)
Fig. 3: Effect of polyunsaturated oils (FLO and FIO) and AuNPs on lipid profile in different experimental groups.

- Represents the mean value ± S.E. (n=10 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. (P < 0.05)
- Represents significant difference between control group and treated group using student's unpaired t-test, ¥ (P < 0.0001), ¶ (P < 0.001), # (P < 0.01) and € (P < 0.05).
- Represents significant difference between HFD group and treated group using student's unpaired t-test, ᴫ (P < 0.0001), ** (P < 0.01) and * (P < 0.05).

Fig. 4: Effect of polyunsaturated oils (FLO and FIO) and AuNPs on relative weight of heart (RW Heart) value in different experimental groups.
(a±) Represents the mean value ± S.E. (n=10 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. (P < 0.05)

($) Represents significant difference between control group and treated group using student's unpaired t-test, ¥ (P < 0.0001), ¶ (P < 0.001), # (P < 0.01) and € (P < 0.05).

($) Represents significant difference between HFD group and treated group using student's unpaired t-test, ¥ (P < 0.0001), ** (P < 0.01) and * (P < 0.05).

Fig. 5: TS of Heart of (a) normal control rats shows the normal the normal histological structure of cardiac myocytes, (b) HFD control rats' shows intermyocardial oedema dispersed the muscle fibers far away from each other associated with inflammatory cells infiltration, (c) Rats fed HFD 50% + FLO 10% revealed intermyocardial oedema and few intermuncular inflammatory cells infiltration, (d) Rats fed HFD 50% + FIO 10% showed no histopathological changes, (e) Rats fed HFD 50% + FLO 10% + AuNPs showed no histopathological changes, (f) Rats fed HFD 50% + FIO 10% + AuNPs showed no histopathological changes. (H&E, scale bar 20.00μm, magnification ×400)

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AI: Antherogenic index</td>
<td>DHA: Docosahexaenoic acid</td>
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<td>ALA: α-linolenic acid</td>
<td>EPA: Eicosapentaenoic acid</td>
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<td>AuNPs: Gold nanoparticles</td>
<td>FIO: Fish oil</td>
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<td>CHD: coronary heart disease</td>
<td>FLO: Flaxseed oil</td>
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<td>CVD: Cardiovascular diseases</td>
<td>H&amp;E: Hematoxylin and Eosin</td>
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<td>RW: Relative weight</td>
<td>SPR: Surface Plasmon Resonance</td>
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<td>rpm: round per minute</td>
<td>TEM: Transmission electron microscopy</td>
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Conclusion

The study concluded that mixtures of PUFAs oils and AuNPs could have the ability to lower weight gain in obese rats and relieved the various biochemical and histological abnormalities resulted due to obesity metabolic disorders.

References

16. Turkevich, J., Stevenson, P. C. and Hillier, J., A study of the nucleation and growth processes in...
43. Hu, F. B., Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. Obesity reviews, 14(8), 606-619 (2013).


**References**


