بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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التوثيق الإلكتروني والميكروفيلم

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Effect of Chitosan and Gold Nanoparticles on Drug Metabolizing Enzymes in the Liver of Adult Male Rats

A Thesis

Submitted for the award of the degree of Ph.D. in Zoology

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2020

Abstract

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Keywords: CYP450; Drug metabolizing enzymes; Drug interactions; Oxidative stress; liver enzymes; Histopathology changes.

There is limited available information about the effect of nanoparticles on drug metabolizing enzymes (DEMs). Changes in DMEs could result in serious drug interactions. Drug interaction is responsible for the high rate of mortality, withdrawal of many drugs from the market, and high economic burden. Therefore, this study aimed to investigate the effect of exposure to chitosan and gold nanoparticles and the possible drug interactions they could elicit on hepatic phase I and II DMEs, the liver functions and integrity, oxidative damage and liver architecture in male rats. Animals were divided into three equal groups; the first group was control, groups 2 and 3 were treated with chitosan nanoparticles (200 mg/kg, 50±5 nm) and gold nanoparticles (4 mg/kg, 15±5 nm), respectively. Rats were administered their respective doses orally and daily for 10 days. Both chitosan and gold nanoparticles decreased the body weights by more than 10%. Gold nanoparticles caused significant reductions in the gene expression of *CYP1A1*, *CYP2E1*, *CYP3A4*, quinone oxidoreductase1 *(NQO1),* and carboxylesterase (*CE*), and elevated the expression of *CYP2D6,* microsomal epoxide hydrolase *(mEH),* and N-acetyltransferase2 *(NAT2).* Chitosan nanoparticles elevated the expression

of *CYP2E1*, *CYP2D6*, and *mEH* and reduced that of UDP-glucuronosyltransferases1A1 *(UDPGT1A1*). For most enzymes, the changes in the gene transcript levels were parallel to the changes in the protein levels of these enzymes. Gold nanoparticles reduced the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) as well as reduced glutathione (GSH) level, and elevated the malondialdehyde (MDA) level in liver. Both gold and chitosan nanoparticles disturbed the architecture of liver, but the deleterious effects after gold nanoparticles treatment were more prominent. Taken together, gold nanoparticles severely perturbed the DMEs and would result in serious interactions with many drugs, herbs, and foods. Knowledge of these interactions must be disseminated among the health practitioners. Precautions must be taken with the new products containing gold nanoparticles especially by those with chronic diseases.

Introduction

Nanoparticles (NPs) are materials in which the basic unit in three-dimensional space falls within the range of the nanometer scale (1-100 nm) or within that range of at least one dimension **(Khan *et al.*, 2019).** Nanotechnology is one of the most important areas of active analysis in science of materials **(Darroudi *et al.*, 2013; Zak *et al.*, 2013).** NPs have emerged as a novel alternative to overcome the multidrug resistance of bacteria encountered globally due to antibiotic misuse. Use of nanoparticles as antimicrobial could resolve mechanisms of bacterial resistance, as the microbicidal nature of nanoparticles result from direct contact with the bacterial cell wall, without penetration into the cell **(Wang *et al.*, 2017).**

Nanoparticle chitosan has gained growing interest due to its biocompatibility, biodegradability, high permeability, cost-effectiveness, non-toxic property and excellent film forming ability. Moreover, its ability to enhance the penetration of large molecules across a mucosal surface and its recognition as muco adhesivity chitosan **(Saini *et al.*, 2010; Ghadi *et al.*, 2014).**

Among various nanoparticles (NPs), gold nanoparticles (AuNPs) constitute promising candidates in biomedicine to be applied in diagnostic aid **(Boisselier and Astruc 2009),** drug and gene delivery **(Pissuwan *et al.*, 2011),** and photothermal cancer therapy **(Kong *et al.*, 2008; Malugin and Ghandehari 2010).** AuNPs based delivery systems are being widely explored for use in cancer chemotherapy treatment as they offer increased drug efficacy with low toxicity to healthy tissue, high biocompatibility, along with versatile production methods, which enable custom

design **(Ujfalussy *et al.*, 2003).** At least one product of this type is in clinical trials **(Libutti *et al.*,**

2010). AuNPs have thus been predicted to have a promising future in mainstream clinical practice **(Pissuwan *et al.*, 2011).**

Drug metabolizing enzymes (DMEs) are a diverse group of proteins that are responsible for metabolizing a vast array of xenobiotic chemicals, including drugs, carcinogens, pesticides, pollutants, and food toxicants, as well as endogenous compounds, such as steroids, prostaglandins, and bile acids **(Coon, 2005 ; Brown *et al.*, 2008 ; Rendic and Guengerich 2010).**

Cytochrome P450 (CYP) enzymes play a major role in the metabolic clearance of the majority of xenobiotics, including approximately 80% of the marketed drugs and drugs of abuse such as alcohol, tobacco, and methamphetamine, Although metabolism of therapeutic drugs decreases their bioavailability, in turn leading to a decrease in their efficacy, metabolism of these drugs also aids in decreasing toxicity **(Kumar, 2010).**

The liver safeguards xenobiotics from entering the blood and eventually entire body. However, CYP-mediated metabolism also produces reactive oxygen species (ROS) and reactive metabolites, leading to increased oxidative stress and cellular toxicity. Therefore, high dose and/or increased frequency of the use of drugs as well as drugs of abuse cause the liver damage and liver-associated diseases **(Kumar et al., 2012; Fromenty, 2013).**

AIM OF THE STUDY

The present study was carried out to investigate the effect of chitosan and gold nanoparticles on the mRNA expression of hepatic phase I and phase II drug metabolizing enzymes in adult male rats enzymes such as cytochrome P450 1A1, 3A4, 2D6, and 2E1, microsomal epoxide hydrolase, quinone oxidoreductase, UDP-glucuronosyltransferases1A1 (UDPGT1A1), Carboxylesterase, N-acetyltransferase, antioxidant enzymes, biochemical parameters and histopathological changes.

2. Review of Literature

2.1. Nanotechnology

The emergence of nanotechnology in the last three decades has changed the perception of drug discovery and development by opening many hidden doors in disease pathophysiology and treatment options **(Peer *et al.*, 2007).** Nanotechnology allows for the understanding and control of materials at the atom and molecule scales. Over recent years, several advantages have been attributed to nanotechnology, of which the following are the most important; the size of the nanostructured materials has a range between 1 and 100 nm, the physical and chemical properties can be controlled through the manufacturing technique parameters at a molecular level, and the final nano-sized structures can be connected in order to attain larger constructs. Nanotechnology has been intensely used in nanomedicine, which uses nanoscaled materials for the prevention, diagnosis, and treatment of diseases **(Bamrungsap *et al.*, 2012; Safari and Zarnegar 2014).**

2.1.1. Nanoparticles

Nanoparticles (NPs) have developed a reputation for their ability to deliver drugs specifically to their site of action **(Tammam *et al.*, 2015).** Some NPs formulations are investigated clinically for treatment of cancer. Nanoparticles solve many of the biopharmaceutical and pharmacokinetic problems associated with many drugs in a variety of diseases. NPs boost the therapeutic efficiency of ionised drugs; improve the penetration of water soluble compounds, proteins, peptides, vaccines, siRNA, miRNA, DNA and other biological therapeutics. Surface modification of

nanoparticles with targeting ligands makes the drug delivery system much versatile and can selectively deliver at target site **(Sperling and Parak 2010).** The pharmaceutical companies are taking advantage of nanotechnology which gives new life to those drugs that were previously considered unmarketable due to low solubility and bioavailability, and high toxicity and marked side effects **(Onoue *et al.,* 2014).**

Therefore, and with the widespread use of NPs in our daily life, investigating the possible toxic effects of NPs is a major concern. **Paur *et al*. (2011)** reported that nanotoxicology focuses upon gaining a thorough understanding of the relationship between the toxicity of NPs depending on their dose levels and physicochemical properties such as size, shape and reactivity material composition. **Lin *et al*. (2008) and Sarkar *et al*. (2014)** described that nanoparticles are known to induce reactive oxygen species (ROS) production, leading to oxidative stress.

Additionally, another consideration is the form of administration of nanoparticles that could have an effect on their toxicity. Injection of nanoparticles through the tail vein has been documented to cause lower toxic effects than those administered through intraperitoneal and oral routes **(Zhang *et al.,* 2010).**

2.1.2. Chitosan nanoparticles

Chitosan is an amino polysaccharide derived from deacetylation of chitin of arthropods and insects exoskeleton and is considered a dietary fiber due to its indigestibility by digestive enzymes **(Kohda *et al.,* 2012).** Chitosan nanoparticles (CNPs) exhibit more superior activities than chitosan and have been reported to have higher immune-enhancing effect, anticancer activity, and antimicrobial activity than those of chitosan. In

addition, nanoparticles possess a stronger curvature of the surface, compared to large particles; this produces more dissolution pressure with a corresponding increase in solubility **(Patel and Agrawal 2011).**

Chitosan nanoparticles are natural materials with excellent physicochemical, antimicrobial and biological characteristics, which make them superior environment-friendly materials. CSNPs were shown to pose no harmful effect on humans **(Yang *et al.*, 2014; Manikandan and Sathiyabama 2016; Ilk *et al.*, 2017).** CSNPs, due to their small size and large surface area to volume ratio, are favorable carriers for different drugs specially hydrophobic drugs in cancer drug delivery application **(Rajan and Raj 2013).** CSNPs also participate in the reduction and stabilization processes during the synthesis of other types of nanoparticles **(Tiwari *et al.*, 2011).** CSNPs have gained growing interest due to its biocompatibility, biodegradability, high permeability, cost-effectiveness, non-toxic properties, and excellent film forming ability. Moreover, CSNPs have the ability to enhance the penetration of large molecules across a mucosal surface **(Ghadi *et al.*, 2014).** Although the safe use of CSNPs is still an area of debate, many studies consider chitosan a safe polymer that can be employed in several *in vivo* applications **(Vunain *et al.*, 2017).**

2.1.3. Gold nanoparticles

Gold nanoparticles (AuNPs) are the most widely studied nanomaterials for biomedical applications due to the finely controlled shape, size, and surface chemistry **(Li *et al.*, 2014).** The organ distribution of gold nanoparticles depends on size, whereas small gold nanoparticles

of 5-15 nm have a broader organ distribution than large gold 50-100 nm nanoparticles **(Chen *et al.*, 2009).** At sizes larger than 5 nm, gold is assumed to be chemically inert. However, the chemical reactivity of AuNPs for diameters less than 3 nm is most likely different than larger gold nanoparticles **(Tsoli *et al.*, 2005).** AuNPs exhibit unique chemical and physical properties, such as their ease of synthesis in a variety of sizes (between 1 and 100 nm) and

shapes (spheres, nanoshells, rods, hollow, prims, diamonds) **(Conde *et al.*, 2016).** AuNPs have intense light absorption and scattering, high photothermal conversion rate, biocompatibility, and ease of surface functionalization with different molecules (nucleic acids, proteins, and drugs) (**Figure 2.1**). Gold nanoparticles with a long diffusion period have been shown to accumulate in the liver and spleen and to have a direct effect on gene expression **(Cho *et al.*, 2009).** Gold nanoparticles have been broadly applied to various biomedical fields such as biosensing assays and clinical diagnosis. AuNPs are emerging as promising therapeutic agents for treatment of AIDS **(Bowman *et al*., 2008)**, tumors **(Kennedy *et al*., 2011)**, and Parkinson's disease **(Kogan *et al.*, 2006).**

Nanotechnologies have developed stable AuNPs, which show antioxidant properties by scavenging free radicals **(Dkhil *et al.*, 2015).** Promising potency of AuNPs in treatment of inflammatory and autoimmune diseases has been reported **(Leonavičienė *et al.*, 2012).** *In vitro* studies using mouse cells revealed that AuNP induced proliferation of keratinocytes, activation of phagocytes, expression of pro-inflammatory cytokines, and maturation of neutrophils **(Yen *et al.*, 2009).**

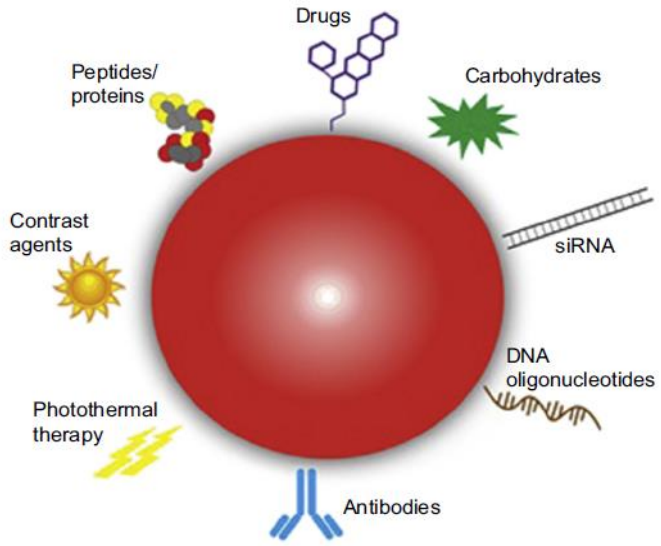

Figure 2.1: Schematic overview of the possible functionalization of gold nanoparticles (AuNPs) **(Carvalho *et al*., 2019).**

Moreover, the oral administration of AuNP to mouse increases the cell proliferation and the secretion of pro-inflammatory cytokines **(Małaczewska, 2015).** Administration of 10 nm AuNP for 3 and 7 days to rats caused a significant decrease in SOD levels in liver **(Abdelhalim *et al.*, 2015).** These changes could result from the damage of the tissues caused by AuNPs, which interfere with the antioxidant defense structure, thereby probably giving rise to (ROS) formation, which may ultimately lead to cell death **(Nel *et al.*, 2006; Abdelhalim and Jarrar 2011).** Spherical gold nanoparticles with a variety of surface modifiers were shown to be toxic to human cells such as skin HaCaT keratinocytes **(Wang *et al.*, 2008).**

2.2. Hepatotoxicity of nanoparticles

Nanoparticles were found to reach the systemic circulation and disseminate to several organs such as the liver **(Jain *et al.*, 2008).** Nanoparticles have been found to be accumulated in liver which means that nanoparticles could be transported to the organs after uptake by the gastrointestinal tract **(Liu *et al.*, 2009; Ma *et al.*, 2009).** The biological responses to toxic substances and distribution pathways may be different depending on the doses accumulated in the body. The nanoparticles when ingested into the body can be distributed to different regions because of their small size. They can cross the intestine and further distributed into the blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach **(Hillyer and Albrecht 2001).**

Ballou *et al.* (2004) described that most nanoparticles that accumulate in liver and spleen cause serious side effects. For instance, cadmium selenide (CdSe) quantum dots remained in the tissue for up to eight months and caused hepatotoxicity. Upon exposure to a mixture of proteins, NP associate with proteins and form protein corona. This alters the surface chemistry, an adsorbed protein conformation, and the subsequent biological responses, cellular NP uptake, catalytic activity of cytochrome P450 (CYP) enzymes toward drugs **(Ajdari *et al.*, 2017; Choi *et al.*, 2017; Choi and Joo 2018).**

2.3. Drug metabolizing enzymes in the liver

The liver is a vital organ that is essential to sustaining life in many organisms due to its numerous functions. The liver produces proteins including those involved in blood clotting. The liver is also responsible