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Gold nanoparticles ameliorate acetaminophen induced hepato-renal injury in rats

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ABSTRACT

Valuable effects of gold particles have been reported and used in complementary medicine for decades. The aim of this study was to evaluate the therapeutic efficacy of gold nanoparticles (AuNPs) against acetaminophen (APAP) induced toxicity. Albino rats were administered APAP at a dose of 2 g/kg *p.o.* once only. After 24 h of APAP intoxication, animals were treated with three different doses of AuNPs (50 μg/kg, 100 μg/kg, 150 μg/kg) orally or silymarin at a dose of 50 mg/kg *p.o.*, once only. Animals of all the groups were sacrificed after 24 h of last treatment. APAP administered group showed a significant rise in the AST, ALT, SALP, LDH, cholesterol, bilirubin, albumin, urea and creatinine in serum which indicated the hepato-renal damage. A significantly enhanced LPO and a depleted level of GSH were observed in APAP intoxicated rats. Declined activities of SOD and Catalase, after acetaminophen exposure indicated oxidative stress in liver and kidney. The activities of ATPase and glucose-6-Phosphatase were significantly inhibited after APAP administration. AuNPs treatment reversed all variables significantly towards normal level and was found nontoxic. Thus it is concluded that gold nanoparticles played a beneficial role in reducing acetaminophen induced toxicity and can be used in the development of drug against hepatic as well as renal diseases, after further preclinical and clinical studies.

1. Introduction

In modern material sciences nanotechnology is one of the most active research fields. Nanotechnology is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early detection, treatment, and prevention of diseases. Gold nanoparticles (AuNPs) are among the most studied metal nanoparticles due to their promising properties such as biocompatibility, easy synthesis, and facile surface modification (Geckeler and Nishide, 2009). Thus, the interest in AuNPs is continuously increased. Due to the chemical stability and good biocompatibility AuNPs are used for variety of medical applications including diagnostics, drug delivery, and biosensing (Cao et al., 2011; Xia et al., 2011; Pissuwan et al., 2011; Upadhyayula, 2012). AuNPs possess antiangiogenic, anti-inflammatory (Mukherjee et al., 2005; Chen et al., 2012) and antioxidant activities (Miyachi et al., 1987; BarathManiKanth et al., 2010). AuNPs have successfully been used to treat rheumatoid arthritis (Tsai et al., 2007) and plaques of Alzheimer's disease (Health News; 12:10, 2006). AuNPs were found effective in reducing cancer masses (Huang et al., 2007).

Liver disease is a worldwide health problem. Synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. More than 900 drugs have been found to induce liver toxicity, which is a leading cause of acute liver failure in the United States (Navarro and John, 2006). Viral hepatitis, chronic alcohol consumption, and nonalcoholic fatty liver disease are the most common causes of liver disease worldwide. All these conditions generate liver injury and inflammation, thereby activating liver fibrogenesis, which can progress to cirrhosis and the life-threatening complications of liver failure and portal hypertension, as well as to incident hepatocellular carcinoma (Lotersztajn et al., 2005).

Acetaminophen (also known as paracetomol) has been available since the 1950s as an over-the-counter product for pain and fever relief. APAP has long been recognized as potentially lethal because of dose-related hepatic and \renal injury (Boyer and Rouf, 1971). APAP hepatotoxicity is the classical example of direct

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liver injury. APAP overdose induces nephrotoxicity which occurs in approximately 1–2% of patients (Mazer and Perrone, 2008). APAP toxicity is associated with increased level of hepatocellular enzymes viz., AST, ALT, LDH and SALP into circulation (Manokaran et al., 2008; Bhadauria and Nirala, 2009). APAP intoxication inhibits the activity of antioxidant enzymes (SOD, CAT, GPx, GR, G6PDH, AND GST) (Gupta et al., 2006; Manokaran et al., 2008; Sabina et al., 2009).

This study was aimed to evaluate the therapeutic aspect of AuNPs against APAP toxicity. To the best of our knowledge this is the first study to show hepatic as well as renal protective effect of pure AuNPs against APAP toxicity. Pure colloidal gold is tasteless and non-toxic relative to gold salts which accumulate in the body and cause toxic side effects (Abraham and Himmel, 1997). World Health Organization also approved gold as a food additive in 1983. It has been proposed that most of toxicities associated with the use of gold aurothiolates were probably due to the gold trichloride formed in vivo by disproportionation (Abraham and Himmel, 1997). However no evidence showed that pure colloidal gold causes toxicity at the clinical, histological, cellular and molecular levels (Chen et al., 2012). According to Perrelli and Piolatto (1992) metallic gold is excreted mainly through kidneys. We choose 3–5 nm nanogold, that can be efficiently excreted through kidneys, and the nanogold does not accumulate inside the body.

2. Materials and methods

Female albino rats of *Wistar* strain (160 ± 10 g body wt) were used in this study. Animals were housed under standard husbandry conditions (25 ± 2 °C temp, 60–70% relative humidity and 12 h photoperiod) and had access to standard rat feed and drinking water ad libitum. Animals were treated and cared in accordance with the guidelines recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

2.1. Preparation of gold nanoparticles

Gold nanoparticles (3–5 nm) were manufactured by Gold NanoTech, Inc., Taipei, Taiwan. AuNPs were produced with physical vapor deposition (PVD) process which maintains 99.99% purity of gold nanoparticles, and the unique technology was applied to allow our AuNPs to be evenly dispersed in sterilized water. Therefore, unlike nanogold made with chemical reduction which requires the addition of dispersing agent to avoid the aggregation of nanoparticles, the gold nanoparticles used in this study are evenly suspended in water without addition of dispersing agent. This further increases the purity of nanogold.

2.2. Chemicals

Acetaminophen (APAP) was procured from Smithkline Beecham, (Batch no. 0103). Silymarin and other chemicals were procured from Sigma-Aldrich Company, Ranbaxy, New Delhi and Himedia Laboratories Ltd. Mumbai, India. All diagnostic kits were procured from E-Merck, auto analyzer (Micro Lab 200, Merck) was used for the measurements.

2.3. Preparation of doses and treatments

A suspension of APAP (2.0 g/5 ml/kg) was made in hot distilled water and administered orally according to Nirala and Bhadauria (2008). Colloidal solution of AuNPs were prepared in distilled water and different doses of AuNPs (50, 100 & 150 μg/5 ml/kg *p.o.*) were administered to the animals orally. Silymarin (50 mg/5 ml/kg, p.o.) was prepared in 1% gum acacia and silymarin was given as positive control (Nirala and Bhadauria, 2008).

2.4. Experimental procedure

Animals were divided in seven groups of six animals each. Group I served as control, Group II was administered AuNPs at a dose of 150 μg/kg *p.o.* once only and served as AuNPs *per se*. Group III- VII were administered (APAP) at a dose of 2 g/kg *p.o.* once only. Group III served as experimental control (APAP *per se*). After 24 h of APAP administration, group IV–VI were treated with (AuNPs) at three different doses (50, 100 & 150 μg/kg *p.o.*) once only and animals of Group VII were administered silymarin at a dose of 50 mg/kg *p.o.* once only, as a standard drug. Animals of all groups were sacrificed after 24 h of the last treatment.

2.5. Blood biochemistry

Blood samples were collected from retro-orbital venous sinus. Blood samples were allowed to stand at room temperature for 30 min and serum was harvested by centrifugation at 2000 rpm for

Table 1
Protective effect of AuNPs against APAP induced alterations in hepatospecific markers.

Treatments	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	SALP (mg Pi/h/100 ml)
Control	70 ± 3.86	37.78 ± 2.08	42 ± 2.32	201 ± 11.11
AuNPs *per se*	68.4 ± 3.78	38 ± 2.10	44 ± 2.43	200 ± 11.05
APAP *per se*	250 ± 13.82[#]	325 ± 17.96[#]	166 ± 9.17[#]	559 ± 30.90[#]
APAP + AuNPs 50 μg/kg	122 ± 6.70[*]	125 ± 6.9[*]	85 ± 4.6[*]	310 ± 17.1[*]
%protection	(71.1%)	(69.6%)	(65.3%)	(69.5%)
APAP + AuNPs 100 μg/kg	95.16 ± 5.26*	69.9 ± 3.86[*]	65 ± 3.5[*]	250 ± 13.8[*]
%protection	(86%)	(88.8%)	(81.4%)	(86.3%)
APAP + AuNPs 150 μg/kg	95 ± 5.25[*]	68.5 ± 3.78[*]	63 ± 3.48*	245 ± 13.5[*]
%protection	(86.1%)	(89.3%)	(83.0%)	(87.7%)
APAP + Sily 50 mg/kg	92 ± 5.08[*]	60.5 ± 3.34[*]	58 ± 3.2[*]	238 ± 13.1[*]
%protection	(87.7%)	(92.0%)	(87.0%)	(89.6%)
F Value (at 5% level)	95.94[@]	207.90[@]	100.37[@]	65.141[@]

Data are mean ± S.E.; N = 6.
Abbreviations: APAP = Acetaminophen; AuNPs = Gold nanoparticles: Sily = Silymarin; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; LDH = Lactate dehydrogenase; SALP = Serum alkaline phosphatase.
[@] Significant at 5% for ANOVA.
[#] APAP vs Control.
[*] APAP + Therapy vs APAP at $P \leq 0.05$.