



Protective Effect of Propolis Against Hepatic Histological and Histochemical Alterations Induced by Naked Gold Nanoparticles

Mansour I. ALMANSOUR¹*, Mosaid A. ALFERAH² & Bashir M. JARRAR³

¹ Department of Zoology, College of Science, King Saud University, Saudi Arabia

² Department of Biology, College of Science and Arts at Onizah, Qassim University, Saudi Arabia

³ The Research Group program, Deanship of Scientific Research, King Saud University, Saudi Arabia

SUMMARY. Very little, if any, is known about propolis protective effect against the toxicity induced by gold nanoparticles (GNPs). The present investigation was conducted to explore the protective role of propolis against the toxicity induced by GNPs on the hepatic tissues. Male Wistar albino rats were exposed to 10 or 20 nm GNPs at a dose of 2000 µg/kg together with or without propolis for 15 days. Hepatic biopsies from all rats were subjected to histological and histochemical examinations. Exposure to GNPs has induced hepatocyte cytoplasmic vacuolation, hydropic degeneration, sinusoidal dilatation, Kupffer cells hyperplasia, pyknosis, inflammatory cells infiltration and glycogen depletion. Propolis demonstrated partial hepatoprotectivity against hydropic degeneration, glycogen depletion and inflammatory cells infiltration but showed no protection for the hepatic tissues from nuclear alterations, Kupffer cells hyperplasia and sinusoidal dilatations. In conclusion, the findings may reveal hepatoprotective effect of propolis against some alterations induced in the hepatic tissues by GNPs toxicity due to its antioxidant properties.

RESUMEN. Muy poco se conoce acerca del efecto protector de propóleos contra la toxicidad inducida por nanopartículas de oro (PNB). La presente investigación se llevó a cabo para explorar el papel protector del propóleo sobre la toxicidad inducida por el PNB en los tejidos hepáticos. Ratas macho albinas Wistar fueron expuestas a 10 o 20 nm de PNB a una dosis de 2000 mg/kg con o sin propóleos durante 15 días. Las biopsias hepáticas de todas las ratas fueron sometidas a exámenes histológicos e histoquímicos. La exposición a PNB indujo vacuolización citoplásmica en hepatocitos, degeneración hidrópica, dilatación sinusoidal, hiperplasia de las células de Kupffer, picnosis, infiltración de células inflamatorias y agotamiento de glucógeno. Propóleos demostró hepatoprotección parcial contra la degeneración hidrópica, el agotamiento de glucógeno y la infiltración de células inflamatorias, pero no demostró protección para alteraciones nucleares de los tejidos hepáticos, hiperplasia de células de Kupffer ni dilataciones sinusoidales. En conclusión, los resultados revelan el efecto hepatoprotector de propóleo contra algunas alteraciones inducidas en los tejidos hepáticos por toxicidad PNB debido a sus propiedades antioxidantes.

INTRODUCTION

Gold nanoparticles have been widely investigated in cancer therapy, radiotherapy, photothermal therapy, diagnosis, drug delivery, and immunohistochemistry¹⁻⁴. Fine GNPs have unique surface and optical properties that make them biologically active with long blood circulating time and affinity to accumulate in the vital organs mainly liver, kidney and spleen⁵.

It has been suggested that the toxic effects of GNPs might be related to their surface area, shape, size and charge where smaller particles are more reactive and more toxic than the larger

ones⁶⁻¹¹. These fine particles may induce oxidative stress and interact with DNA and proteins that could result damage to tissues, cells and macromolecules^{12,13}.

Naked GNPs of 5-20 nm were found to be more toxic and had wider organ distribution than the larger ones with liver was being as a target organ¹⁴⁻¹⁹. Hepatic tissues receive high blood flow and have high exposure to small GNPs with long circulating residue in comparison to larger particles exposure^{9,10}. Some studies reported toxic effects of GNPs in the tissues of the vital organs including the liver^{15-19,20}.

KEY WORDS: Antioxidant, Gold nanoparticles, Hepatic tissues, Nanotoxicity, Propolis.

* Author to whom correspondence should be addressed. E-mail: cnrgsa@gmail.com

Propolis is a mixture of wax with natural resinous substances collected from plants by honeybees (*Apis mellifera*) and able to keep the hives free of germs, exclude draught and protect against invaders^{21,22}. This bee glue is known for its antioxidant properties due to its phenolic and essential oils contents. Propolis has been used for centuries as antimicrobial, anti-oxidative, anti-ulcer, hypotensive agent and immune system stimulant^{23,24}. Furthermore, propolis is being invested highly in cosmetic applications and marketed by pharmaceutical industry due to its antioxidant capacity and ability to combat lipid peroxidation in the liver^{25,26}. Propolis was also reported to have role in tissue regeneration and blood capillaries strengthening²⁷. In addition, it was found to have protective effects from hepatotoxicity caused by carbon tetrachloride, acetaminophen, and inorganic toxicity²⁸⁻³⁰.

The present work was conducted to investigate the effect of propolis against the histological and histochemical alterations induced in the hepatic tissues by GNPs

MATERIALS AND METHODS

Experimental Subjects

Sixty male Wistar albino rats of 12 weeks age and weighing 210-230 g were obtained from the animal house (College of Pharmacy, King Saud University, Saudi Arabia). The rats were randomly assigned and separately caged to five test groups and a control one (10 rats each) with access to food and water ad libitum.

Gold nanoparticles

Spherical naked GNPs (10 and 20 nm) were purchased from Sigma-Aldrich, USA.

Propolis

Propolis crude manufactured by Marnys Spanish Company to Saudi Arabian Drug Store Ltd was used. An aqueous extract of this crude was prepared from capsules, each contained 1000 mg.

Experimental protocol

The animals were handled and all experiments were conducted in accordance with the protocols approved by King Saud University ethical committee. The rats were exposed to GNPs together with propolis as follows:

Group I: received neither GNPs nor propolis but a single intraperitoneal injection of 100 µL of the vehicle for consecutive 15 days.

Group II: received a daily intraperitoneal in-

jection of 100 µL GNPs of size 10 nm at a dose of 2000 µg/kg for consecutive 15 days.

Group III: received a daily intraperitoneal injection of 100 µL GNPs of size 20 nm at a dose of 2000 µg/kg for consecutive 15 days.

Group IV: received a daily intraperitoneal injection of 100 µL GNPs of size 10 nm at a dose of 2000 µg/kg, before being exposed to a single dose of propolis (15mg/kg) for consecutive 15 days.

Group V: received a daily intraperitoneal injection of 100 µL GNPs of size 20 nm at a dose of 2000 µg/kg, before being exposed to a single dose of propolis (15 mg/kg) for consecutive 15 days.

Group VI: received a daily oral dose of propolis (15 mg/kg) for consecutive 15 days.

Sample preparation

All members of all groups were euthanized by cervical dislocation after 15 days of treatment. Fresh liver biopsy from each rat of all groups were cut rapidly, fixed in neutral buffered formalin, dehydrated with ascending grades of ethanol (70, 80, 90, 95, and 100%), cleared in 2 changes of xylene before being impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (4-5 µm) of the control and GNPs treated rats were stained according to Pearse³¹ with hematoxylin and eosin stain, Mallory trichrome stain, Periodic Acid-Schiff (PAS) method and Prussian blue reaction.

RESULTS

Liver of control rats

Microscopic examination of the control rats liver revealed normal hepatocytes, normal hepatic portal spaces and normal lobular architecture together with normal hepatocytes glycogen content (Figs. 1A-C). Bile duct hyperplasia and hemosiderin precipitation were not demonstrated in the liver of all control rats.

Liver of rats treated with GNPs

The following abnormalities were seen in the hepatic tissues of rats exposed to GNPs.

Hepatocyte cytoplasmic vacuolation

Hepatocytes mild cytoplasmic vacuolation was seen together with partial cytoplasmic clearing and swelling in rats exposed to 10 nm and to lesser extent in those exposed to 20 nm GNPs (Fig. 2A).

Hydropic degeneration

Cytoplasmic hydropic degeneration was ob-

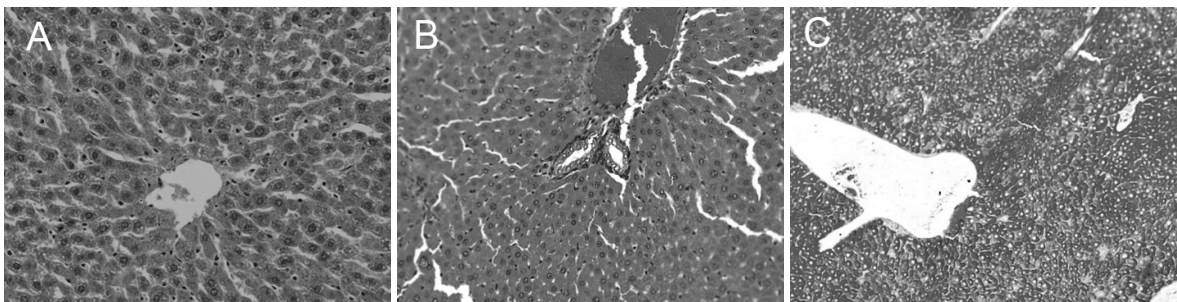


Figure 1. Light micrographs of sections in the liver of control rats received single intraperitoneal injection of 100 μ L of GNPS vehicle for 15 days demonstrating normal hepatocytes and normal lobular architecture (A), normal hepatic portal space (B), and normal glycogen content (C).

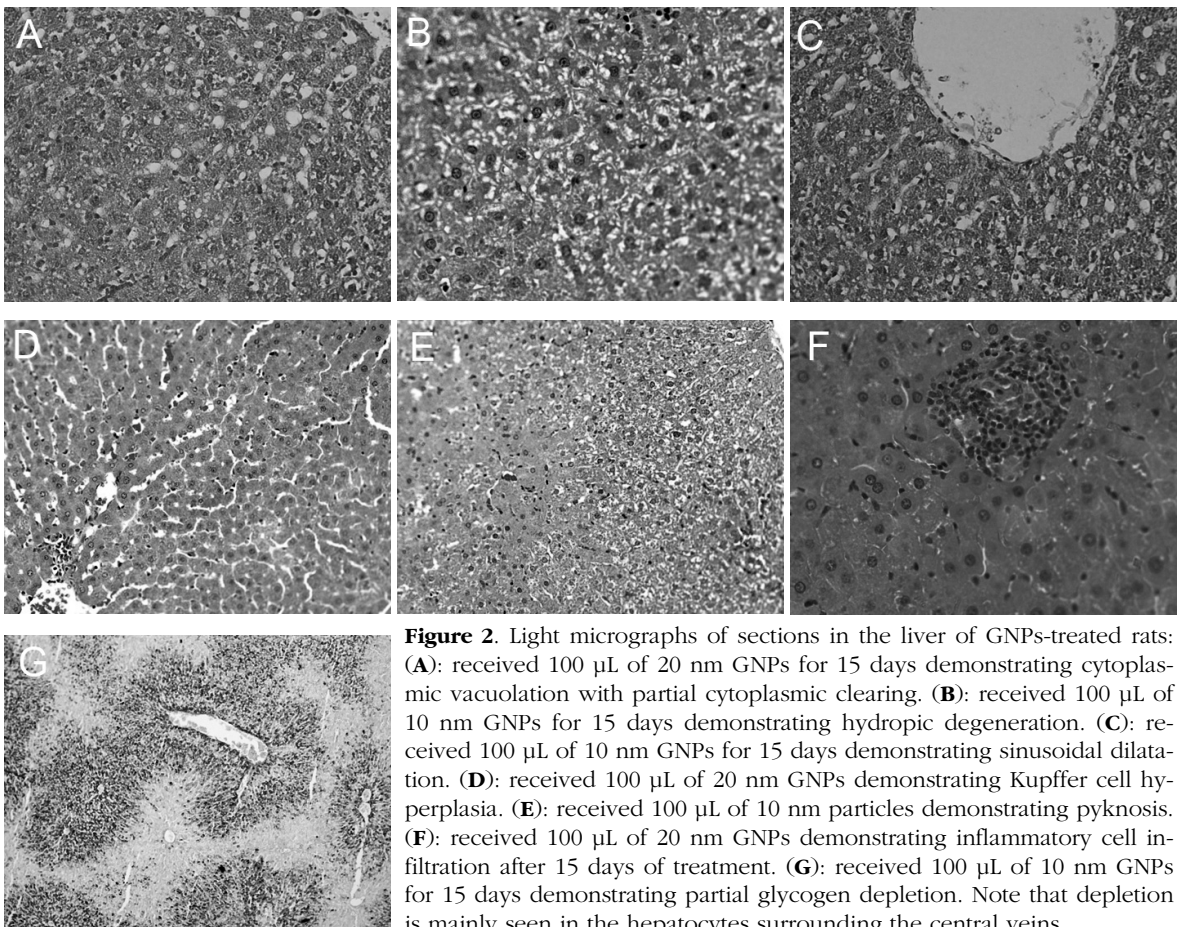


Figure 2. Light micrographs of sections in the liver of GNPs-treated rats: (A): received 100 μ L of 20 nm GNPs for 15 days demonstrating cytoplasmic vacuolation with partial cytoplasmic clearing. (B): received 100 μ L of 10 nm GNPs for 15 days demonstrating hydropic degeneration. (C): received 100 μ L of 10 nm GNPs for 15 days demonstrating sinusoidal dilatation. (D): received 100 μ L of 20 nm GNPs demonstrating Kupffer cell hyperplasia. (E): received 100 μ L of 10 nm particles demonstrating pyknosis. (F): received 100 μ L of 20 nm GNPs demonstrating inflammatory cell infiltration after 15 days of treatment. (G): received 100 μ L of 10 nm GNPs for 15 days demonstrating partial glycogen depletion. Note that depletion is mainly seen in the hepatocytes surrounding the central veins.

served in the hepatocytes of rats exposed to GNPs for 15 days (Fig. 2B). This alteration was more prominent in rats exposed to 10 nm GNPs than those treated with 20 nm ones.

Sinusoidal dilatation

The liver of this group of rats exhibited sinusoidal dilatation after 15 days of GNPs exposure. This vascular alteration was characterized by widening of capillaries lining the hepatic strands (Fig. 2C).

Kupffer cells hyperplasia

Enlargement and activation of Kupffer cells appeared in rats treated with 20 nm GNPs and was more prominent in rats exposed to 10 nm GNPs (Fig. 2D).

Nuclear alterations

Some hepatocytes showed condensed chromatin materials with irregular nuclear border after 15 days of treatment with both 10 nm and 20 nm GNPs (Fig. 2E). The pyknotic hepatocytes

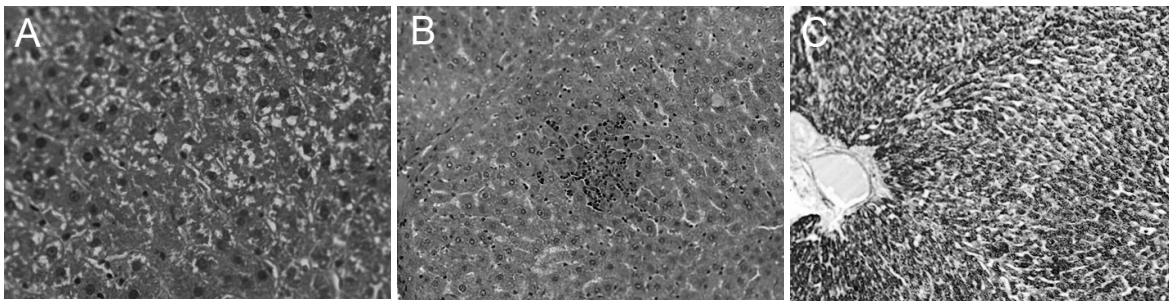


Figure 3. Light micrographs of sections in the liver of rats: **(A)**: received combined 10 nm GNPs with propolis for 15 days demonstrating less hydropic degeneration in comparison with those received GNPs only. **(B)**: received combined 20 nm GNPs with propolis for 15 days demonstrating less pronounced inflammatory reaction in comparison with those received GNPs only. **(C)**: received combined 20 nm GNPs with propolis for 15 days demonstrating more glycogen content in comparison with the liver of rats subjected to GNPs only but still less evident than the control rats. **(D)**: received combined 10 nm GNPs with propolis for 15 days demonstrating Kupffer cells hyperplasia and sinusoidal dilatation almost similar to that demonstrated by rats received GNPs only.

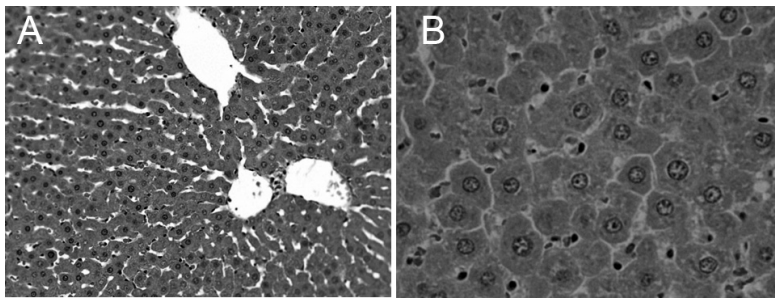


Figure 4. Light micrographs of sections in the liver of rats were subjected to a daily oral dose of propolis (15 mg/kg) for 15 days demonstrating normal architecture and zonal accentuation **(A)** together with normal hepatocytes, Kupffer cells and sinusoids **(B)**.

were also severely affected by cytoplasmic alterations.

Inflammatory cells infiltration

Focal scattered necrotic nodules infiltrated with inflammatory cells mainly macrophages appeared in the liver of rats exposed to 20 nm GNPs for 15 days. More macrophages infiltration was seen in hepatic tissues of animals exposed to 10 nm GNPs (Fig. 2F).

Glycogen depletion

Periodic acid-Schiff (PAS) stain indicated partial glycogen depletion in the hepatocytes of animals received GNPs for 10 days and to lesser extent in those exposed to 20 nm GNPs (Fig. 2G). Depletion was more prominent in the hepatocytes surrounding the pericentral areas while those surrounding the portal spaces were less affected. Bile duct hyperplasia was not identified in the hepatic tissues of all rats exposed to GNPs while Prussian blue reaction showed no indication of hemosiderin precipitation in liver of these rats.

Liver of rats treated with GNPs plus propolis

Hydropic degeneration and inflammatory reaction improvement was detected in the hepatic tissues of rats received GNPs plus propolis for 15 days than those were subjected to GNPs only (Figs. 3A and 3B). Moreover, glycogen content in the hepatocytes of rats received GNPs plus propolis for 15 days was more pronounced in comparison of that seen in rats exposed to GNPs alone (Fig. 3C). In addition, Kupffer cells hyperplasia, sinusoidal dilatation and pyknosis, were still evident in rats treated with GNPs plus propolis (Fig. 3D).

Liver of rats treated with propolis only

Microscopic examination of the liver sections of all members of this group demonstrated well preserved intact lobular architecture and zonal accentuation as well as normal hepatocytes, sinusoids and hepatic portal triads (Figs. 4A and 4B).

DISCUSSION

Gold nanoparticles have been widely used in drug delivery, medical imaging, biological sensors and hold promise in diagnostic and therapeutic purposes of a wide spectrum of disorders^{1-4,32,33}. A considerable number of studies were carried out on the potential toxicity of GNPs concerning shape, charge and size^{15-19,34}. Previous studies demonstrated that small, rod-shaped and positively charged GNPs were more toxic than larger, spherical and ionic ones respectively⁶⁻⁹. Other studies showed that GNPs may reveal a high risk potential on liver and other vital organs^{15-19,34}.

The results of the present study may indicate that propolis afford protection against hydropic degeneration, necrosis, inflammatory cells infiltration and glycogen depletion in the hepatic tissue of rats exposed to 10 nm or 20 nm GNPs combined with propolis. This protection might be due to the antioxidant activity of propolis against oxidative stress in the liver induced by GNPs. These findings are in line with some reports where propolis demonstrated hepatoprotectivity and therapeutic potential against several chemical and environmental toxicants³⁵⁻³⁸. The antioxidative capacity of propolis might be related to its pharmacological and biological contents such as flavonoid, phenolic acid esters, terpenes, cinnamic acid and others^{21,24,26}. Furthermore, propolis has the ability to increase the activity of some antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase together with suppressing cytochrome p-450 enzymes^{22,25,29,39}. Propolis combats lipid peroxidation that impairs cellular structure and function^{35,39} and can also inhibit membrane free radical formation and protects the mitochondria against oxidative damage^{35,39-43}.

The protective role of propolis against inflammatory cells infiltration with predominance of macrophages as seen by the results of the present study might be due to the immunostimulant and immunomodulating activity of this bee glue. Macrophages stimulate immune cells to respond to foreign substances and to participate in regeneration function. The predominance of macrophages might indicate a compensatory response to facilitate clearance of cellular debris accumulated due to GNPs toxicity.

In addition, the findings of the present study may indicate that propolis could not restore hepatocytes nuclear severity towards normal and to protect against pyknosis induced by

GNPs. This might reveal that propolis could not compensate against nuclear alterations induced by GNPs.

CONCLUSIONS

It can be concluded from the findings of the present work that propolis has inhibitory effect against some hepatic alterations induced by GNPs oxidative damage. In addition, the results of the present study may indicate that propolis has hepatoprotective potential suggesting its support therapy for those who are exposed to GNPs.

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