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Research Article

Zoology

The potential therapeutic effect of loaded ginger nanoparticles against Ehrlich ascites carcinoma (EAC) bearing mice-induced renal toxicity

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KEY WORDS

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ABSTRACT

Ehrlich ascites carcinoma (EAC) are undifferentiated, natural mouse mammary adenocarcinomas that are primarily used for research. The current study aims to assess whether loaded ginger nanoparticles have a therapeutic effect against renal toxicity induced by EAC in mice. This study employed forty-nine mice, which were separated into seven groups as follows: Group1(GR1) was utilized as the negative control. EACs were implanted in GR2 mice. The GR3 was treated with Cis (40 µg/mouse) on the first day post-inoculation. The fourth, fifth, and sixth treatment groups received Gin, AGNPS, and Gin/AGNPS (0.4 mg/mouse for 6 days). The seventh treated group was injected with Cis (40 µg/mice) and then administered with Gin/AGNPS for 6 days. All mice were slaughtered to examine the biochemical, histological and immunohistochemical alterations in the kidney. The findings demonstrated a substantial increase in the EAC group's overall body weight. However, in contrast to (GR2), groups that received Cis, AGNPS, Gin, Gin/AGNPS, and Cis/Gin/AGNPS displayed a small percentage increase in body weight. Furthermore, EAC produced renal impairment, as demonstrated by increase in serum urea and creatinine levels. While the treatment group with Gin/AGNPS and Cis/Gin/AGNPS improved and reduced these differences in renal function stress. Histological and immunohistochemical staining verified these results. Present results imply that loaded ginger nanoparticles may possess anti-oxidant, anti-cancer, and renal protective qualities in addition to inhibiting the damage that EAC causes to the kidney dysfunction.

Introduction

One of the major, frightening, and popular health issues of the twenty-first century remains cancer. In women, the most widespread malignancy globally is breast cancer, impacting one out of every seven women at some point in their lives and accounting for the majority of fatalities because of cancers **(Judasz et al., 2022)**. The most common and efficient cancer treatment is chemotherapy, and it is for the majority of cancer types **(Riddell, 2011)**. Chemotherapy's primary strategy is to specifically target tumor cells. It is known as a systemic therapy due to its ability to circulate all across the organism and eliminate malignancies that have dispersed to areas other than the primary tumor. This sets it apart from other medications but this affects cancer cells as well as regular cells, resulting in hepato, nephro, neuro, oto, and cardio-toxicity, which is induced in part by the generation of reactive oxygen species (ROS) (**Oun et al., 2018; American Society, 2019)**. Physicians employ cisplatin to treat several types of solid malignancies. It fights cancer in several different ways. Even though the percentage of initial responses is regularly elevated, cisplatin therapy often leads to chemotherapy resistance and unsuccessful treatment (**Galluzzi et al., 2012**). It damages normal tissues in addition to cancerous ones. In particular, it affects kidney tissues and can lead to acute impairment and chronic kidney disease **(Tang et al., 2022)**. Cisplatin causes acute kidney injury that has a complex pathophysiological map associated with cellular uptake, efflux, apoptosis, vascular injury, oxidative stress, and inflammation. Despite pharmaceutical interventions and clinical trials, there is no consistent and stable pharmacological therapeutic approach for preventing acute kidney damage in cisplatin-treated patients **(McSweeney et al., 2021)**.

Nanotechnology has been identified as a potential alternative for improving existing cancer treatments or providing unique therapeutic methods. Utilizing nanoparticles (NPs) in cancer therapy research can accomplish significant objectives such as minimizing side effects from delivered medications, generating a wide range of medication delivery nano-formulations, and utilizing the electrical, magnetic, or optical properties of NPs to target and destroy tumor cells **(Li et al., 2019 and Riley et al., 2019)**. By addressing the shortcomings of traditional drug administration methods, nanotechnology has become a promising tool in the

medication delivery field **(Sivadasan et al., 2023)**. Recent advances in medical science and nanotechnology have produced an array of nanoparticles and nanomaterials that researchers synthesize either biologically or physiochemically from different large-scale components, including silver, gold, platinum, copper, etc. **(Singh et al., 2018)**. Because of their exceptional qualities, which include anti-cancer, bacterial, fungal, proliferative, inflammatory, and oxidant capabilities. Silver nanoparticles (AgNPs) have a lot of attention in the cancer research field **(Zhao et al., 2023)**. Several investigations have shown that AgNPs reach cells via endocytosis and localize inside endolysosomal component cells and the perinuclear cytoplasm **(Greulich et al., 2011)**. It influences cell respiration and produces ROS. It triggers damage to DNA, mitochondria, oxidative stress, and apoptosis inside malignant tissues **(Sukirtha et al., 2012)**.

Ginger (*Zingiber officinale*) is a medicinal and health-promoting plant that is commonly utilized in food and pharmaceutical products. Its therapeutic qualities are prominent from its raw extract **(Fakhri et al., 2021)**. It has a wide range of bioactive components, such as gingerol, zingerone, parasols, and shogaols **(Kandemir et al., 2018)**, which consist of phenolic compounds, terpenes, lipids, and carbohydrates. **Jafarzadeh et al. (2021)** reported that phenolic chemicals and terpenes are responsible for the majority of the pharmacological effects of ginger. Ginger polyphenols have anticancer effects, including anti-inflammatory, antiproliferative, antiangiogenic, antimetastatic, cell cycle arrest, apoptosis, and autophagy. As a result, consistent ginger consumption aids in the avoidance and management of breast cancer, as well as protecting against chemotherapy side effects of ginger **(Usman et al., 2023)**. Zingerone, a phenolic alkanone that has anti-inflammatory, anti-apoptotic, and antioxidant activities, has the ability to protect the kidneys from cisplatin-induced damage, including inflammation, DNA damage, oxidative stress, apoptosis, and a decrease in renal tissue aquaporin 1 (AQP1) protein levels **(Kandemir et al., 2019)**.

Consequently, the purpose of this work is to evaluate and discuss the data suggesting that loaded ginger nanoparticles could shield mice from EAC-induced renal toxicity, with the ultimate objective of examining the loaded ginger nanoparticles' possible therapeutic advantages in lowering tumor-associated renal toxicity.

Materials and Methods

Materials

Ginger

Ginger rhizomes were obtained from a local market in Tanta, Egypt.

Mice

forty-nine female Swiss albino mice (CD-1 strain) weighing 23 ± 2g and aged 6-8 weeks were obtained from the National Research Center Animal House in Dokki, Giza, Egypt.

Ehrlich ascites carcinoma (EAC)

All EAC cell lines in this investigation were purchased from the Pharmacology and Experimental Oncology Unit at Cairo University's National Cancer Institute in Cairo, Egypt.

Cisplatin

Cisplatin (Cis: Mylan Co., S.A.S. France) was dissolved in phosphate-buffered saline (PBS: Lonza, Bio Whittaker, USA) and frozen at -80 °C until utilized.

Methods

Preparation of ethanolic (80%) extract of ginger

After hand washing and using a sharp knife to peel it, the ginger was dried in a hot air oven at 55°C (horizontal forced air dryer, Faculty of Science, Tanta University, Egypt). The dried ginger powder was finely ground. For one week at room temperature, the ground ginger (20 g) was extracted with 160 ml of 100% ethanol and 40 ml of distilled water for three days. The extract was filtered using cotton gauze and then filter paper, and then the ethanol was evaporated by stirring overnight (**El-Borady et al., 2020**). As a crude extract, the ginger extract obtained after ethanol evaporation was used.

Experimental maintenance of mice

Female mice had been habituated for two weeks before experimentation and randomly assigned to experimental groups (n = 7 per group), including a control group. They were kept on a consistent light and dark cycle and fed a conventional pellet diet with unlimited tap water.

Loading of ginger extract on synthesized silver nanoparticles (AgNPs)

- The AgNPs were synthesized following the chemical reduction method according to **Mohsen et al. (2020)**, and the resultant AgNPs were kept in a dark place at the standard temperature for further usage.
- The synthesized AgNPs were mixed with the same quantity of ginger extract and stirred for 6h at 37°C. The solution was then stored in a closed, dark glass bottle until used **(Mohsen et al., 2020)**.

In vitro cytotoxicity assay of synthesized nanoparticles

Cell lines and culture Formation

This study used mammary gland breast cancer (MCF-7) cell lines. ATCC provided the cell line through the Holding Company for Biological

Products and Vaccines (VACSERA) in Cairo, Egypt.

Cytotoxicity (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) assay

The MTT test was employed in accordance with **Denizot and Lang (1986)** to assess the impact of ginger, AgNPs, and ginger loaded on AgNPs with or without cisplatin on tumor cell growth and calculation of the cell viability using the equation developed by **Cory et al. (1991)**.

Detection of IC_{50} and *in vivo* LD_{50} of ginger and manufactured nanoparticles

The FDA's most recent data referred to the IC_{50} of manufactured nanoparticles as the concentration at which 50% of MCF-7 cell populations perish. IC_{50} values were calculated using the dose-response curve, which plotted nanoparticle concentrations (µg/ml) on the X axis and cell growth inhibition percentage on the Y axis. In vivo LD_{50} values in mg/kg (body weight) of the synthesized nanoparticles. Using this formula: log (LD_{50}) = 0.435 x log (IC_{50}) + 0.625 **(Halle, 1998 and Spielmann et al., 1999)**.

Experimental sublethal dose used in this study

The sublethal doses of each synthesized nanoparticle, including ginger were estimated, considering the mean weight of the mice.

Experimental design

In 42 naive female mice, 2.5×10^5 EAC cells were administered intraperitoneally (IP). The next day, EAC-bearing mice received therapy with sublethal dosages of ginger extract and manufactured nanoparticles with or without cisplatin, with cisplatin alone serving as a positive control besides the negative control.

GR 1: Injected (i.p.) with saline and was used as a negative control group. **GR 2**: Used as a naïve tumor group. **GR 3**: Treated with Cisplatin (40 µg /mouse) (**Adawy A. 2020**) on the first day as a positive control. **GR 4**: Treated with Ginger (0.4 mg/mouse) for 6 days. **GR 5**: Treated with AGNPs (0.4 mg/mouse) for 6 days. **GR 6**: Treated with Gin/ AGNPs (0.4 mg/mouse) for 6 days. **GR 7:** Treated with Cis (40 µg /mouse)/ Gin/ AGNPs (0.4 mg/mouse) for 6 days.

On the 12th day after EAC inoculation, mice were slaughtered via cervical dislocation. Biochemical assays, histological and immunohistochemical examinations were all performed. Before scarification, blood was extracted from the retro- orbital plexus and placed in heparinized microhematocrit tubes.

Assessment of the changes in the body weight

The starting and final body weights were measured. The following formula was

used to get the change in body weight: [(final b.wt. - initial b.wt.) / initial b.wt] × 100 is the percentage of b.wt. change.

Analysis of kidney function (Creatinine & Urea serum)

Labbe et al. (1996) method was applied to quantify level of creatinine. While, urea level was determined by **Tiffany et al. (1972)** technique.

Histological examination of kidney sections

Paraffin sections of the kidney were cut 5-µm in thickness by rotary microtome and stained with routine hematoxylin and eosin according to **Bancroft and Gamble (2002).**

Immunohistochemistry investigation

Caspase 3 antibodies (1:50 dilution) were provided by DAKO, Carpinteria, CA, for immunohistochemical labeling of 5-µm formalin-fixed, paraffin-embedded sections. using the **Bressenot et al. (2009)** technique.

Statistical analysis

To find significant differences between treatments, a one-way analysis of variance (ANOVA) was carried out. Tukey post hoc tests between groups were carried out, if there was a significant difference in means. Means without a shared letter differ considerably (Tukey's test, $p < 0.05$). Every group receiving treatment was assessed in relation to the controls using Dunnett's test. Paired examples t-test was employed to compare each group's starting and final body weight. For all statistical tests P value > 0.05 was considered not statistically significant P value < 0.05 was considered statistically significant. P value < 0.01 was considered statistically moderately significant. P value < 0.001 was considered statistically highly significant. All data were analyzed using IBM SPSS Statistics for Windows, Version 27, Graphpad Prism V. 8.3, and Microsoft Excel 365 **(Microsoft Corporation, USA).**

Ethical aspects

All animal experiments were carried out strictly in accordance with the guidelines of the Ethics Committee, Science Faculty, Tanta University, Tanta, Egypt, as well as the guidelines of the Institute of Laboratory Animal Resources, National Research Council (NIH Publications No. 8023, revised 1978) and the international guidelines for animal experimentation. The experimental protocol was approved and assigned a reference number (**IACUC-SCI-TU-0284**). This study had no human samples.

Results

In vitro cytotoxicity MTT assay

All of the investigated substances improved cell growth inhibition in the MCF-7 cell lines in a manner that depends on dosage (Fig.1). These findings show that the studied chemicals

may have a greater effect on tumor cell growth than on normal cells. In vitro IC_{50} values for ginger, AgNPs, and AgNPs/ginger composites were 17.85±1.3 µg/ml, 36.36±2.4 µg/ml, and 13.80±1.1 µg/ml, consecutively.

Calculated in vivo LD_{50} values for the studied compounds (ginger, AgNPs, and AgNPs/ginger composites) were 14.7 mg/kg, 20.13 mg/kg, and 13.2 mg/k, in that order. The experimental doses of the investigated substances were set at 0.4 mg for ginger, AgNPs, and AgNPs/ginger composites.

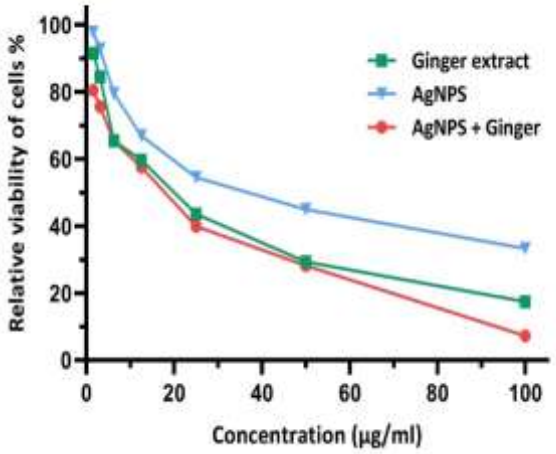


Fig. (1): Relative viability of MCF7 cells with different concentrations in Ginger, AgNPs-loaded with or without Ginger by an MTT assay

Effects of Cisplatin, Ginger, AGNPs and Gin-loaded on AGNPs with or without Cis on body weight

The acquired data reveal remarkable weight changes of mice harboring EAC in contrast to the control group in a time-dependent manner (from day 0 to day 11 post-EAC inoculation), while mice treated with Cis, Gin, AgNPs, AGNPs/Gin, and Cis/AGNPs/Gin showed decreases in weight contrasted with the EAC group, and there is no

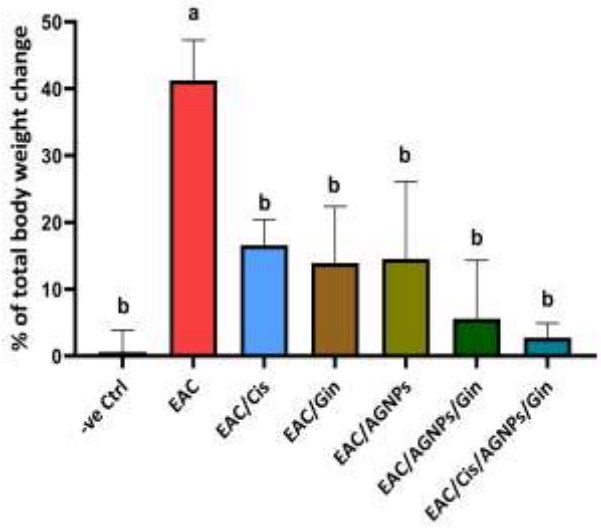
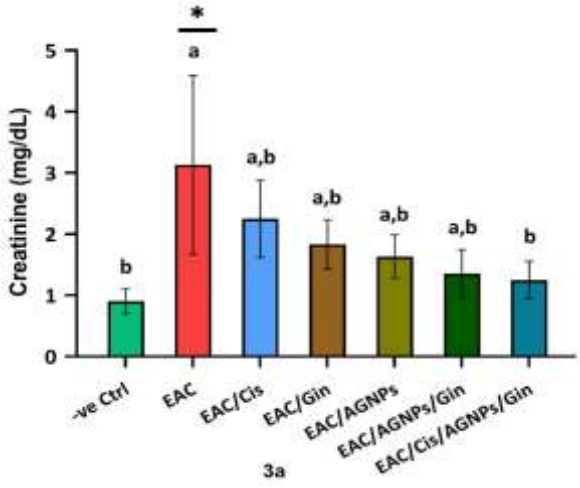


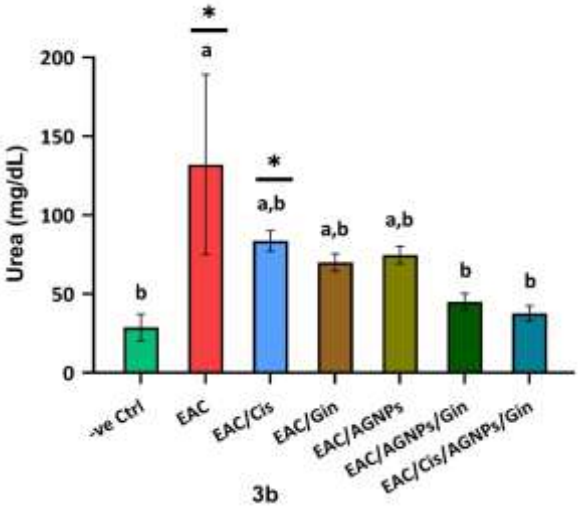
Fig. (2): % of body weight variations between experimental groups
Data represented as Mean ± SD.

Impact of the treatment on the kidney functions among various groups

The level of creatinine displayed a significant superiority in the mice with EACs than the normal. Even so, the treatment with Cis/AGNPs/Gin significantly decreased creatinine compared with the tumor group and showed no difference with the control group. Alternatively, the treatment with Cis, Gin, AGNPs, and Gin/AGNPs had a non-insignificant decrease in creatinine level compared with the group of mice carrying EACs **Fig. (3a)**. The mice in the EACs group significantly increased the serum urea compared with the normal group but the treatment with Gin/AGNPs and Cis/AGNPs/Gin significantly

decreased the urea compared with the EAC-bearing tumor group while showing no variance compared to the control group. Although the treatment with Cis, Gin, and AGNPs non-significantly decreased the urea compared with the EAC-bearing mice group **(Fig. 3 b)**.





Figs. (3a & 3b): Creatinine & urea levels in different experimental groups.
Data represented as Mean ± SD.

Histopathological investigations

The renal cortex of control mice (GR1) seemed normal when examined under a light microscope. Two layers of epithelium encircled the glomerulus, and Bowman's capsule encircled it. The glomeruli had round and oval shapes. Simple cuboidal epithelia lined the proximal convoluted tubules, the cytoplasm of their epithelia was acidophilic, and a profusion of microvilli produced a brush border at the apex. Simple cuboidal epithelium lined the distal convoluted tubules **Fig. (4a)**.

The investigation of the kidney section obtained from EAC-bearing mice showed some histopathological changes that appeared as disorganization and congestion of the glomeruli with uneven Bowman's space as well as swollen and elongated renal tubules with atrophy and degeneration of their lining epithelia, lost their characteristic appearance **Fig. (4b)**. While the kidney section of Cis-treated mice revealed congested glomeruli with widening Bowman's space; mostly renal tubules are normal, and others are degenerated and accumulated with hyaline casts **Fig. (4c)**. However, **Fig. (4d)** illustrates the kidney section of Gin treated group that revealed the renal profile was closely similar to its normal structure, with organized glomeruli with regular Bowman's space with unlimited border; the majority of the renal tubules were regular, and a handful number were degenerated and destroyed. However, treatment with AGNPs showed normal glomeruli with a normal appearance of Bowman's space, a small number of

renal tubules were normal, other tubules have degenerated, and there was a widening of the intertubular septa **Fig. (4e)**.

However, **Fig. (4f)** exhibited an improvement of the renal tissue that is proved by organized glomeruli with ordinary Bowman's space; the vast majority of renal tubules are intact and normal; slightly ones were degenerated and distended, with atrophy of their lining epithelia in EAC-bearing mice treated with AGNPs/Gin. Nevertheless, the kidney section of mice treated with Cis/Gin /AGNPs demonstrated a notable improvement that is represented by healthy glomeruli with normal bowman's space, mostly renal tubules are ordinary, few ones are ruined and their contents are intermixed with each other's **Fig. (4g)**.

Immunohistochemistry examination

Fig. (5 a) illustrated a weak reaction in the Glomeruli and tubules with caspase-3 immunostaining in the kidney of the control mice. In contrast, renal tubules and glomeruli in EAC-bearing mice's kidney section stained with Caspase-3 immunostaining showed strong nuclear and cytoplasmic reactions **Fig. (5b)**. Furthermore, kidney sections treated with Cis showed a modest to moderately favorable response in their renal tubules **Fig. (5c)**. On the other hand, **Fig. (5d)** showed that a small percentage of renal tubules reacted weakly to caspase-3, indicating a markedly reduced caspase-3 immunostain reactivity in the kidney section of the ginger-treated group. But renal tubules and glomeruli in the AgNPs-treated group's kidney section stained with caspase-3 immunostaining showed a moderate nuclear positive reaction for caspase-3 **Fig. (5e)**. Conversely, **Fig. (5f)** revealed a slight cytoplasmic positive reaction for caspase 3 in the renal tubules and a negative reaction in the glomeruli in mice treated with Gin /AGNPs. Nevertheless, Caspase-3 immunostain reactivity was very weak in the kidney section from the Cis/ Gin /AGNPs -treated group (mild positive in the renal tubules and negative reaction in the glomeruli) **Fig. (5g)**.

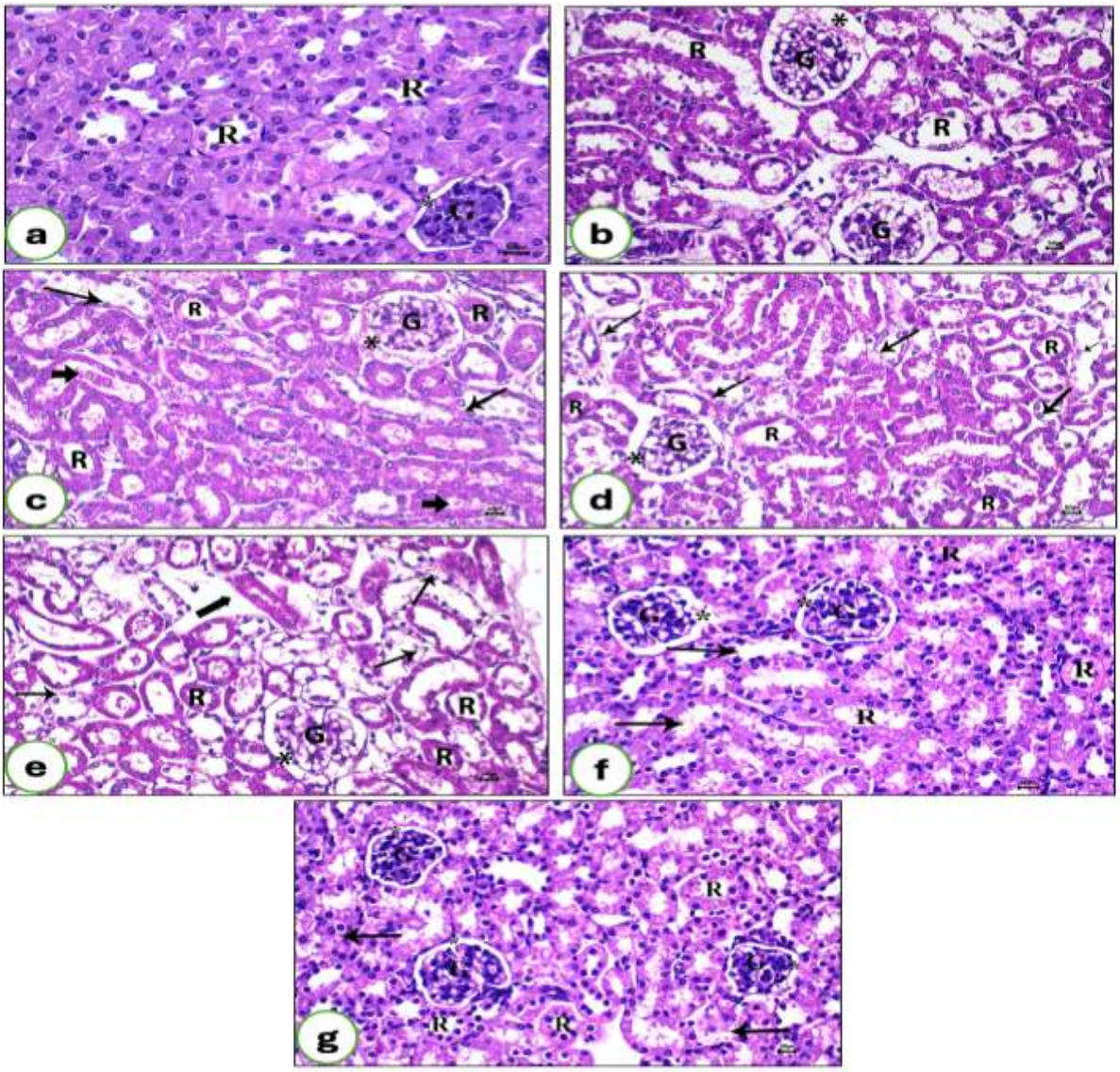


Fig. (4): Photomicrographs of kidney sections of different experimental animal groups stained with H&E **a:** High magnified kidney section of control mice showing organized glomeruli (G) have regular Bowman's space (*) and normal renal tubules (R) (X 400). **b**: High magnified kidney section of EAC mice exhibit disorganized and congested glomeruli (G) with irregular Bowman's space (*), distended and elongated renal tubules (R) with atrophy and degeneration of epithelia that line it (X 400). **C**: High magnified section of cis-treated group showing congested glomeruli (G) with widening Bowman's space (*). The major renal tubules are organized (R), others are degenerated (thin arrows) and accumulated with hyaline casts (thick arrows) (X 400). **d:** High magnified kidney section of the ginger treated group showing regular glomeruli (G) with ordinary Bowman's space with unlimited border (*), mostly renal tubules are normal (R), few numbers are degenerated and destructed (thin arrows) (X 400). **e:** High magnified kidney section of AGNPs treated mice showing healthy glomeruli (G) with organized Bowman's space (*), few numbers of renal tubules (R) are typical, other tubules are degenerated (thin arrows) and widening of intertubular septa (thick arrow) (X 400). **f:** High magnified kidney section of EAC bearing mice treated with AGNPs/Gin (GR6) Showing improvement of the renal tissue that is proved by organized glomeruli (G) with relatively regular bowman's space (*), renal tubules are normal (R), some tubules are degenerated and distended, and their lining epithelia is atrophied (arrows) (X 400). **g:** High magnified kidney section of EAC bearing mice treated with Cis/ AGNPs/Gin (GR7) exhibit pronounced improvement that is represented by normally organized glomeruli (G) with regular bowman's space (*), mostly renal tubules are normal (R), few ones are denaturated, and their contents are intermixed with each other's (Thin arrows) (X 400).

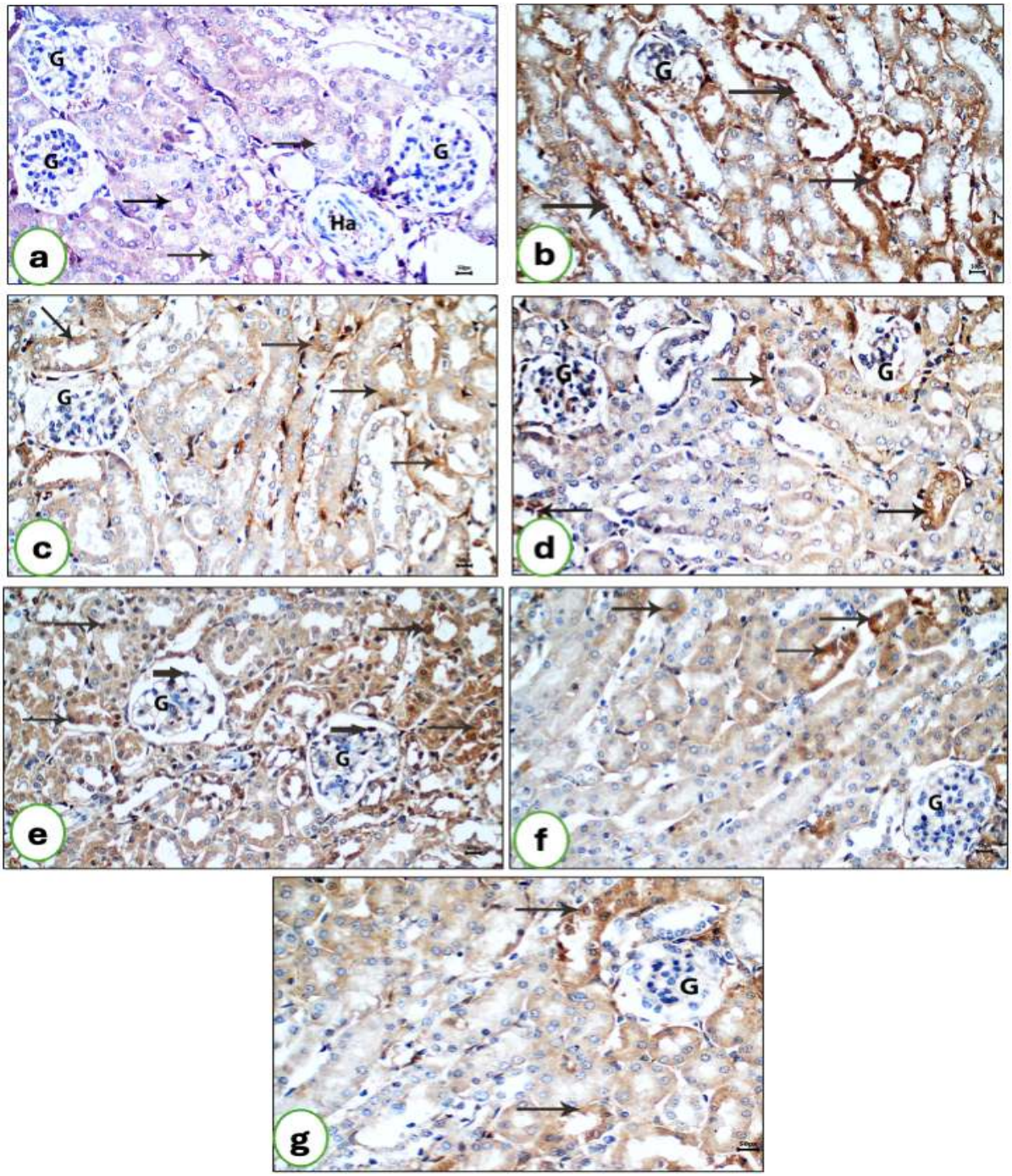


Fig .(5): Photomicrographs of kidney sections stained with caspase 3 immunostain

a: High magnified kidney section of control mice (GR1) showing faint reaction for caspase 3 in the Glomeruli (G) and tubules (arrows) (X 400). **b:** High magnified kidney section of EAC- bearing mice (GR2) exhibit strong positive nuclear and cytoplasmic reactions in the renal tubules (arrows) and glomeruli (G) to caspase 3 stain (X 400). **C:** High magnified kidney tissue of mice that received Cis (GR3) reveal mild to moderate positive expression for caspase 3 immunostain in the renal tubules (X 400). **d:** High magnified kidney tissue of mice received Gin (GR4) showing few numbers of renal tubules (arrows) illustrate significantly decreased caspase 3 immuno-stain reactivity (X 400). **e:** High magnified kidney section of AGNPS treated group (GR5) exhibits moderate nuclear positive reaction for caspase 3 in the renal tubules (arrows) and glomeruli (thick arrow) (X 400). **f:** High magnified section of mice's kidneys received AGNPs/Gin (GR6), showing a mild cytoplasmic positive reaction for caspase 3 in the renal tubules and a negative reaction in the glomeruli (G) (X 400). **g:** High magnified kidney section of EAC- bearing mice treated with Cis /Gin/ AGNPs reveal mild positive reaction to caspase 3 immunostain in the renal tubules (arrows) and negative reaction in the glomeruli (G) (X 400).

Discussion

EAC is a heterogeneous carcinoma that proliferates quickly and has a high transplantation potential **(Gardouh et al., 2020)**. It first originated on its own in a breast cancer mouse model. It is quite sensitive to anticancer medications, much like human breast cancer **(El Gendy et al., 2022).** Several prior studies have relied on Ehrlich's solid tumor (EST) as a trial version to evaluate the anticancer effects of pharmaceuticals and natural components **(Abdeljalil et al., 2023)**. Cancer cells' resistance to anti-cancer medications is a major factor in treatment failure. Although chemotherapy medications increase cancer patients' survival rates, they are known to have several serious adverse reactions that considerably impair the effectiveness of anti-tumor medication and patients' quality of life **(Was et al., 2022)**. Although the platinum-based medications cisplatin, carboplatin, and oxaliplatin are frequently prescribed, their usage is restricted due to significant side effects that limit the dosage **(Oun et al., 2018)**. Using cisplatin affects normal tissues in addition to malignant tissues. Cisplatin is particularly nephrotoxic, causing both acute and long-term kidney **damage (Tang et al., 2022)**. Additionally, cisplatin causes acute kidney injury (AKI) **(McSweeney et al., 2021)**.

Natural products are anticancer, available, and have low side effects. As a result, combining natural components with chemotherapy medications could be a successful anticancer strategy, inhibiting tumor growth and multidrug resistance while reducing chemotherapy drug side effects **(Li et al., 2022)**. Ginger has enormous medicinal properties and health benefits, and both in vitro and in vivo investigations corroborate the impact of gingerol in cancer treatment **(Nafees et al., 2021)**.

Nanoparticles are crucial for improving medication delivery. They can be used as medications and medication delivery apparatuses **(Jain, 2020)**. Ali et al. **(2013)** stated that ginger extract shows promise as a preventive agent against chemotherapy-induced nephrotoxicity, indicating that it may be used in conjunction with chemotherapy to prevent cancer. The ability of nanoparticles (NPs) to modify and combine several physicochemical features to enhance their anti-cancer action is one of the key advantages of using NPs in cancer treatment. Scientists have developed different types of nanomaterials as drug-delivery devices with enhanced bioactivity that are non-toxic and biocompatible **(Dristant et al., 2023)**. Within this research, we combined the anti-tumor effect of ginger with the potent penetrative

AgNPs alongside or without cisplatin to explore the potential therapeutic impact of this composite when administered orally to the mice with EACs.

In the present study, mice carrying EAC had a significant increase in final body weight. These findings were consistent with **Saleh et al. (2022)**. That is because EAC's inflammatory reaction causes progressive tumor growth, which impairs blood and lymph return and increases capillary permeability, resulting in leakage of protein in the abdominal cavity and ascitic fluid accumulation **(Hashem et al., 2020)**. Treatment with cisplatin showed decrease in weight compared to the EAC group. This is consistent with the study, which found that cisplatin reduced the body weight of tumor-bearing mice when compared to the EAC group **(Saleh et al., 2022),** because chemotherapeutic medications can cause anorexia and cachexia **(Donia et al., 2018)**. While the AGNPs/Gin and Cis/AGNPs/Gin treated groups returned to normal control levels. Reduced weight after treatment reflects ginger's anti-proliferative effectiveness, which the nano formulation enhanced. Similarly, prior research found that ginger oils had an antiproliferative effect on cancer **(Alharbi et al., 2023)**.

The present investigation demonstrated that EAC causes impairment in kidney function, as seen by higher levels of serum creatinine and urea than the control group, indicating intrinsic acute renal failure. These findings align with **Saleh et al. (2022)**. Even though improved renal function in EAC-bearing mice treated with cisplatin, urea and creatinine levels remained considerably higher than the control values. Recent investigations have revealed similar results **(Hashem et al., 2020; Arita et al., 2021)**. Nephrotoxicity is a widespread adverse consequence of Cisplatin therapy, and it can be caused by Cis-induced renal tubular obstruction, renal tubule back leaking, or impairment of renal functioning **(Miller et al., 2010)**. Treatment with ginger extract reduced serum creatinine and urea levels. However, treatment with ginger extract loaded on AgNps with or without cisplatin resulted in a significant reduction in serum creatinine and urea levels when compared to the cisplatin-treated group but no difference when compared to the control group. This indicates that ginger extract did not induce any changes or nephrotoxicity. our outcomes are consistent with prior research **(Ali et al., 2013; Alharbi et al., 2023)**.

The histopathological data indicated the kidney sections of the EAC group exhibited disorganized and congested glomeruli with uneven Bowman's space, along with elongated and swollen renal tubules with atrophy and degeneration of their lining epithelia. These changes were consistent with the findings of **Saleh et al.**

(2022). Cisplatin was discovered to cause numerous histological changes in the kidney, including congested glomeruli with enlarging Bowman's space; the majority of renal tubules are normal, while others are deteriorated and aggregated with hyaline casts. These histological changes are consistent with the findings of **Ali et al., (2013)**. The microscopic investigation confirmed the biochemical results, revealing a significant enhancement in kidney tissues in ginger extract loaded on AgNps with or without cisplatin- treated group. The present findings also showed that treatment with ginger extract loaded on AgNps with a combination of cisplatin was more effective than treatment without it, which is partially consistent with recent studies by **Alharbi et al., (2023)**, who proposed that ginger oil may have a synergistic impact with chemotherapy. Caspase-3, an apoptotic protein, triggers caspase-8, resulting in DNA damage and cell death. **(Zargan et al., 2011)**. The EAC group in the current investigation showed a positive response for pro-apoptotic Caspase 3 expressions. Our findings aligned with **Eldaim et al., (2019) who** proved that Ehrlich triggered apoptosis and DNA damage in renal tissues, which agrees with **El-Atrsh et al., (2019)**. The Cis-treated mice showed a noteworthy increase in caspase-3 expression, which was similar to **Saleh et al., (2022)**. **Hashem et al., (2020)** documented the antitumor efficacy of cisplatin and this effect may be attributed to the way cisplatin and DNA molecules interact, which generates free radicals that kill cancer cells **(Saleh et al., 2022)**. Ginger extract is cytotoxic to tumor cells but not to normal cells **(Moheghi et al., 2011)**. A mild positive reaction to caspase-3 indicated that ginger extract could restore the cells' natural state, which was in agreement with the findings of **De Heer et al., (2007)** who stated that the expression of caspase-3 was significantly elevated in cancer tissue compared to the matching normal tissue. The biochemical analyses and histological results that demonstrated a noteworthy enhancement in the renal tissue in mice administered with ginger extract loaded on AgNps with or without cisplatin were corroborated by Caspase-3 **(Khoury et al., 2009)**.

Finally, we concluded that the loaded ginger nanoparticles may possess antioxidant, anti-cancer, and renal protective qualities in addition to inhibiting the damage that EAC causes to the kidney.

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التأثير العلاجى المحتمل لجسيمات الزنجبيل النانونية المحملة ضد سرطان الإيرلش الاستسقائى (EAC) فى الفئران والمسببة للتسمم الكلوي

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سرطان الإيرلش الاستسقائى (EAC) هو سرطان غدي ثديي طبيعي غير متمايز يستخدم في أبحاث الأورام. تهدف الدراسة الحالية إلى تقييم التأثير العلاجى لجسيمات الزنجبيل النانونية المحملة على السمية الكلوية الناجمة عن أورام الإيرلش الاستسقائى في الفئران والفوائد العلاجية المحتملة لجسيمات الزنجبيل النانونية المحملة في تخفيف الضرر الكلوي المرتبط بالورم. تم استخدام عدد تسعاً وأربعين فأراً قسمت إلى سبع مجموعات: الأولى كمجموعة ضابطة سلبية والثانية تم زرع الورم فيها وعولجت الثالثة بالسيسبلاتين (٤٠ ميكروجرام/فأر) في اليوم الأول بعد الحقن بالورم. وتجرعت المجموعات الرابعة والخامسة والسادسة مستخلص الزنجبيل و جسيمات الفضة النانونية والزنجبيل المحمل على جسيمات الفضة النانونية (٤. مليجرام / فأر) على التوالى لمدة ٦ أيام. أما المجموعة السابعة عولجت بـالسيسبلاتين (٤٠ ميكروجرام/فار) ثم تجرعت الزنجبيل المحمل على جسيمات الفضة النانونية (٤. مليجرام / فأر) لمدة ٦ أيام. تم تجهيز عينات الكلى من كل المجموعات لفحص التغيرات البيوكيميائية والنسيجية والكيميائية النسيجية المناعية. أظهرت النتائج زيادة كبيرة في وزن الجسم الإجمالي للمجموعة الحاملة للورم وعلى النقيض أظهرت المجموعات المعالجة بالسيسبلاتين وبمستخلص الزنجبيل وجسيمات الفضة النانونية والزنجبيل المحمل على جسيمات الفضة النانونية مع او بدون السيسبلاتين نسبة مئوية قليلة في زيادة وزن الجسم الإجمالي مقارنة بالمجموعة الحاملة للورم. بالإضافة إلى ذلك أنتج الورم اختلال فى وظائف الكلى كما اتضح من ارتفاع مستويات اليوريا والكرياتينين فى الدم. في حين أن المجموعة المعالجة بالزنجبيل المحمل على جسيمات الفضة النانونية مع السيسبلاتين أظهرت تحسناً واضحاً في وظائف الكلى وفى التركيب النسيجي و الفحص الكيميائي النسيجى المناعي الذى يوضح الموت المبرمج للخلايا الذى يحدثه الورم. وخلصت هذه النتائج إلى أن جزيئات الزنجبيل النانونية المحملة تمتلك خصائص مضادة للأكسدة وللسرطان وللتلف الكلوي بالإضافة إلى تثبيط الضرر الكلوى الذي يسببه الورم.