

Impact of Poria Cocos Nanoparticles Extract Against Ehrlich Solid Tumour Induced Toxicity, Oxidative Stress and Apoptosis in Female Mice Kidney

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The field of cancer nano therapeutics is quickly evolving, and it is being used to address a number of issues with traditional drug delivery techniques. The goal of this study was to find out more about the impact of novel bioactive Poria-loaded sun flowers nanoparticles (Poria Nps) as anti-carcinogenic agent for Ehrlich solid tumour (EST). A total number of 40 adult female mice were divided into 4 groups included control group, Poria Nps group, EST group, and EST treated with Poria Nps group. EST induced toxicity, apoptosis and oxidative stress while treatments of EST with Poria Nps improved this alteration in kidney functions and structure. Moreover, Poria Nps could scavenge free radicals producing beneficial effects against EST induced renal toxicity through activation of oxidative stress and apoptosis. The constructed novel oral nanoparticles developed have promising features in vivo as well as a high level of safety for efficient cancer treatment. Poria cocos nanoparticles (Poria Nps) kill the cancer cells through apoptosis which thereby regulates the proliferation of cancer cells and inhibits its spread to other organs.

Keywords: Apoptosis; Cancer Nano therapeutics; Ehrlich solid tumor; Renal toxicity.

Cancer is the disruption of the healthy cell cycle, which leads to uncontrolled cell proliferation and a lack of differentiation, which are recognized as malignant growths. The most common form of cancer, breast cancer, is also the second leading cause of mortality among women.^{1,2} Ehrlich tumour was mainly characterized as a spontaneous mouse mammary adenocarcinoma

that mimicked breast cancer.³⁻⁵ Ehrlich solid tumours are undifferentiated solid tumours that are widely employed in chemotherapeutic research and tumour studies.^{6,7}

Along with causing disruptions in some tissues of the tumour host, tumour development can modify the form and function of the liver and kidneys.^{8,9} According to research, oxidative stress

and the production of reactive oxygen species by malignancies cause tissue damage and DNA deterioration.^{10,11}

In reality, the idea of chemoprevention using substances derived from nature is gaining more and more attention, especially because conventional cancer therapies have a poor therapeutic record and a high rate of adverse effects.¹² In their simplest forms as syrups, infusions, and ointments, medicinal herbs from the plant kingdom have long been utilized as herbal remedies.¹³⁻¹⁵ The primary medical resource used in traditional or folk medicine, according to the World Health Organization, are medicinal plants.¹⁶

In many of Asian countries, *Poria coco* have a long history of medical use. A *Poria coco* contains triterpenes, steroids, polysaccharides, choline, amino acids, histidine, and other chemicals.¹⁷ In recent decades, significant progress has been achieved in chemical and bioactive research of *Poria coco* polysaccharides and their derivatives that exhibit many beneficial biological activities including anti-inflammatory, anticancer, antiviral and antioxidant activities.¹⁸ Natural polymeric-based nanomaterials have great potential for developing novel drug carrier designs for specific applications. Furthermore, these nanoparticles have therapeutic properties and can provoke a significant immunological response with high biological safety. Therefore; the goal of this study was to find out more about the impact of novel bioactive *Poria*-loaded sun flowers nanoparticles (*Poria Nps*) as anti-carcinogenic agent for EST induced kidney toxicity and injury.

MATERIALS AND METHODS

We bought sunflower seeds at the neighbourhood market, Alexandria-Egypt. The authentic extraction of *Poria cocus* was purchased from Herbal House Centers Company, Cairo, Egypt.

Preparation of Sunflower lecithin and *Poria coco* nanoemulsion

The extraction of lecithin was produced according to Zorzi *et al.*²¹ method with some modifications. The husk of the crude Sunflower seeds were removed, washed with distil water and dried in oven at 40°C for one hour. The dried seeds were crushed by milling machine.

Convenient volume of 5% vinegar was added to the crushed seeds and blended with homogenizer. The mixture was centrifuged for 20 min at 4000 rpm. The extraction was purified three times by adding 20 ml of vinegar to the sample and incubated at room temperature for 30 min., and the precipitate was dried for 24 hours under vacuum at room temperature. Nano-emulsions were created using a modified Solvent-emulsification evaporation technique. The nanoparticles were collected by ultracentrifugation (Beckman Coulter Life Sciences, Optima Max TL, A95761), at 3000 rpm for 20 minutes, then washed twice with distil water and dried using a lyophilizer (Bioevopeak, Benchttop Freeze Dryer, LYO60B-1S).

Animals

A total of 40 female Swiss albino mice (*Mus musculus*), 8–10 weeks old, weighing 25±2 from Egypt Vaccine Establishment's (EVC) animal house colony were provided standard mice feed and water ad libitum. All the experiments were done in compliance with the guiding principles in the care and use of laboratory animals IACUC – SCI – TU – 00179.

Experimental design and animal groups

Mice were equally divided into six groups (Gp1 – Gp4): Gp1: Control Gp; the mice did not receive any therapy. Gp2: *Poria* nanoparticles Gp (*Poria NPs*); the mice received *Poria NPs* orally (25mg/kg bw/2day) orally for 15 days. Gp3: EST Gp; mice were administered hypodermically with approximately 2,500,000 EAC/mouse weakened in buffer saline to begin EST.³ Gp4: Treated EST with *Poria NPs* (EST+*Poria NPs*) in which mice were administered hypodermically with 2,500,000 EAC cells per mouse to induce tumour (EST) and left for 15 days until the tumour improved, at which point they were cured with *Poria NPs* (25mg/kg bw/2day) orally through stomach tube for 15 days.

Blood and serum samples

Blood samples have been collected aseptically by venepuncture into a dry clean and sterile tube without anticoagulant substances and allow it to clot. Blood samples permitted to stand for 30 min at 4 °C for clotting and then centrifuged for 10 minutes at 3000 rpm. The collected serum was kept at -18° C until it was analysed to determine a blood parameter.

Electrolytes and kidney functions Biomarker

Patton and Crouch²²; Bowers and Wong²³

approaches were used to estimate urea and creatinine levels in sera. Estimation of electrolytes levels (Potassium, sodium, calcium and chloride ions) in sera by employing trade kits (Sensa core electrolyte, India) according to Elmasry *et al.*²⁴ and Gupta *et al.*²⁵

Estimations of oxidative and antioxidants parameters in kidney homogenate

Malondialdehyde (MDA), a lipid peroxidation indicator, was measured in homogenate using the method reported by Mesbah *et al.*²⁶

According to Jollow *et al.*²⁷ and Saggu *et al.*²⁸; glutathione (GSH) and catalase activity in homogenate were measured.

Histopathological assessment

Processing for paraffin sectioning followed the immediate incorporation of 10% buffer neutral formalin solution for one to two days to the kidney and liver tissues. In line with Seyedalipour *et al.*²⁹, the histopathological assessment was enabled by staining the sections with haematoxylin and eosin (H&E).

Apoptotic P53 and anti-apoptotic Bcl2 expressions

In accordance with the protocol designated by Tousson *et al.*^{30,31}; P53 and Bcl2 immunoreactivities of the fixed kidney slices were evaluated using the avidin-biotin complex method.

Statistical analysis

An unpaired *t*-test to evaluate the salient variances among treatment groups enabled the undertaking of statistical investigation where data stayed articulated as average values \pm SE. 0.05 served as the threshold for the biochemical data representing the criterion of statistically significant data. The SPSS - statistical version 21 software

package (SPSS® Inc., USA) was used to carry out all statistical analyses.

RESULTS

Electrolytes and kidney functions Biomarker

Table (1) revealed that; significant ($p < 0.05$) increase in levels of urea, creatinine, K⁺, Cl⁻ and significant ($p < 0.05$) decrease in Na⁺ and Ca⁺⁺ in EST in comparison to control. Furthermore, levels of urea, creatinine, K⁺ and Cl⁻ were significantly ($p < 0.05$) decreased but Na⁺ and Ca⁺⁺ were significant ($p < 0.05$) increase in EST + Poria as contrast with the EST +.

Effects of Poria Nps upon markers of oxidative stress in kidney homogenates

Figure (1) indicated a significant increase in MDA contents and significantly decreased in the GSH and activities of CAT in kidney tissues of EST as compared to control. However, EST+Poria Nps significantly decreased MDA content, while it significantly increased in the content of GSH and the activities of CAT compared to EST.

Kidney injury

Kidney structures with normal glomeruli and renal tubules were demonstrated in control (G1), and treated mice with Poria NPs (G2) (Figures 2A&2B). In contrast, marked inflammatory cellular infiltration, moderate degenerative and, atrophy in tubular cells were observed in kidney in EST (G3) (Figure 2C). Kidney sections in EST+Poria NPs (G4) revealed a moderate atrophy and moderate degenerated in tubular cells (Figure 3D).

Bcl2 immuohistochemical changes in kidney

Recognition and distribution of Bcl2 immunoreactivity (Bcl2) at kidney slices of groups under study were revealed in figures (3&5). Kidney

Table 1. Changes in serum electrolytes and kidney function levels

	Control	Poria Nps	EST	EST+ Poria Nps
Urea (gm/dl)	26.7 [#] ±1.38	28.25 [#] ± 1.65	54.5*±2.96	33.75 [#] *±2.93
Creatinine (gm/dl)	0.52 [#] ±0.18	0.49 [#] ± 0.03	0.98*±0.06	0.64 [#] *±0.07
Na ⁺ (mmol/l)	136.6 [#] ± 8.8	135.7 [#] ±0.37	124.5*±7.1	130.9 [#] *±1.4
K ⁺ (mmol/l)	4.19 [#] ±0.24	4.49 [#] ± 0.06	6.06*±0.54	5.41 [#] * ± 0.08
Ca ⁺⁺ (mmol/l)	1.12 [#] ±0.01	1.02 [#] ± 0.01	0.89*±0.01	1.03 [#] ± 0.03
Cl ⁻ (mmol/l)	101.5 [#] ±8.9	102.1 [#] ± 0.37	116.4*±8.2	103.9 [#] ± 1.03

Data are expressed as mean \pm SE. ([#]) and (*) significant difference compared to control and EST respectively

sections in control, and Poria NPs groups show high positive responses for Bcl2 (grade 5) in glomeruli and renal tubules. While faint positive responses for Bcl2 (grade 1), were observed at kidney slices in EST group. Furthermore, mild

positive responses for Bcl2 (grade 3) were observed at treated EST with Poria NPs group.

P53 immunohistochemical changes in kidney

Recognition and distribution in P53 immunoreactivity (P53) at kidney slices of groups

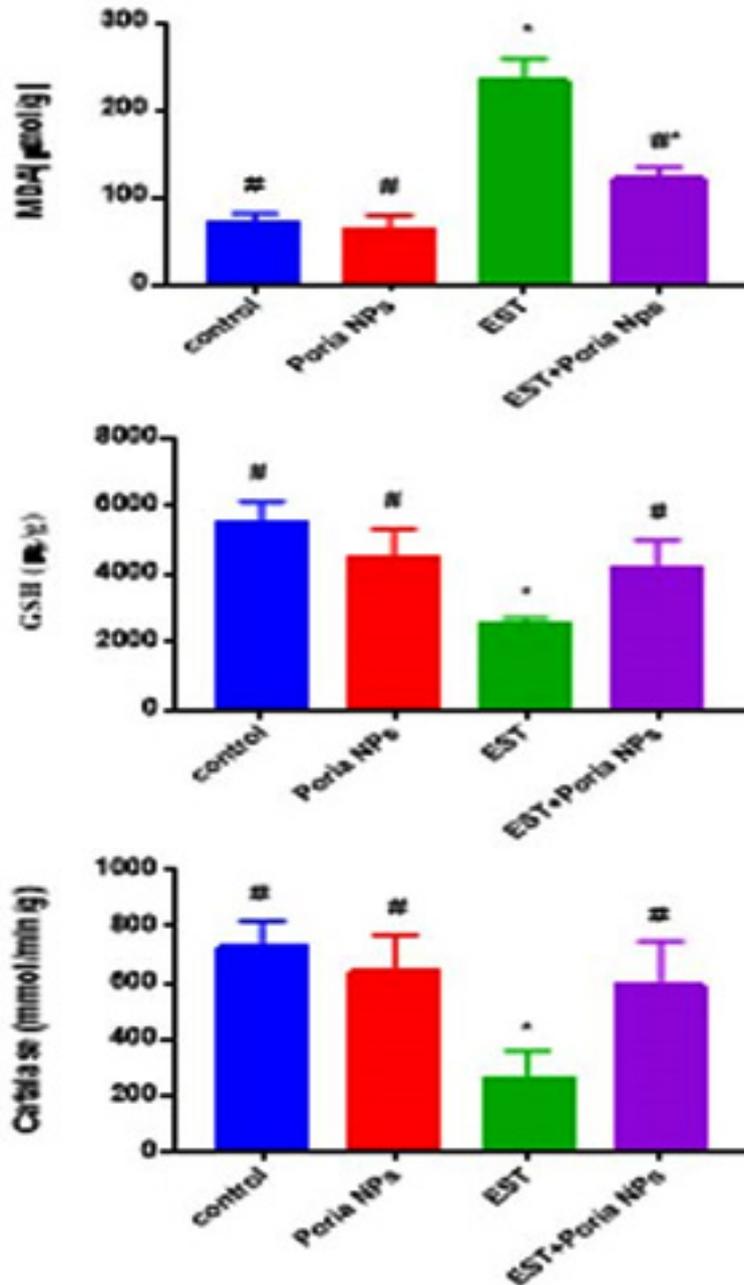


Fig. 1. Changes in Malondialdehyde (MDA), glutathione (GSH) and catalase (CAT) activities in kidney homogenates different groups. Data are expressed as mean ± SE. (#) and (*) significant difference compared to control and EST respectively

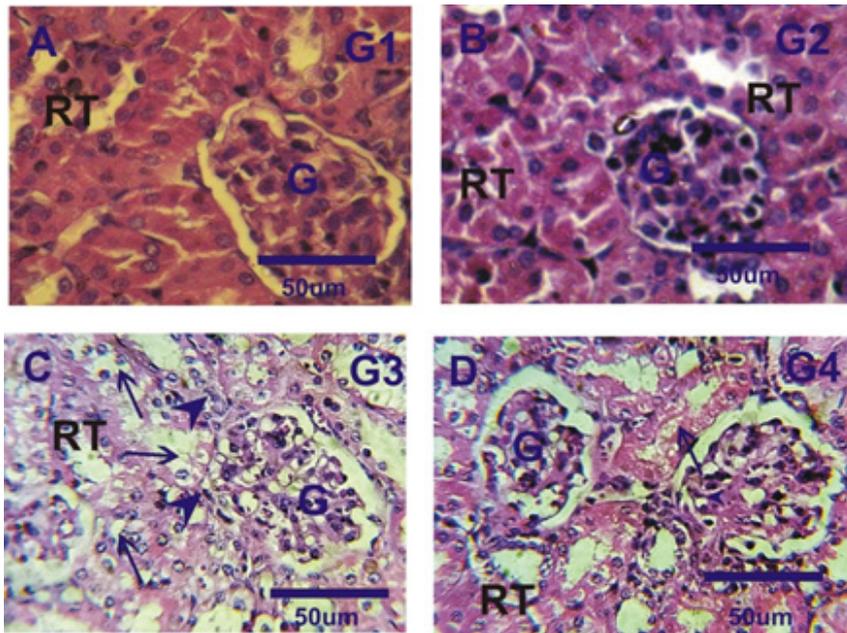


Fig. 2A-2D. Haematoxylin and Eosin-stained photomicrographs of mouse kidney slices from all experimental groups. A, B: Control and Poria NPs-treated groups showed that the glomeruli (G) and renal tubules (RT) had normal histological characteristics. C: Tubular cell atrophy and mild degeneration are shown in kidney sections in EST (arrow heads). D: Moderate tubular cell atrophy and minor urinary tract degeneration were seen in kidney sections from the treated EST with Poria NPs group

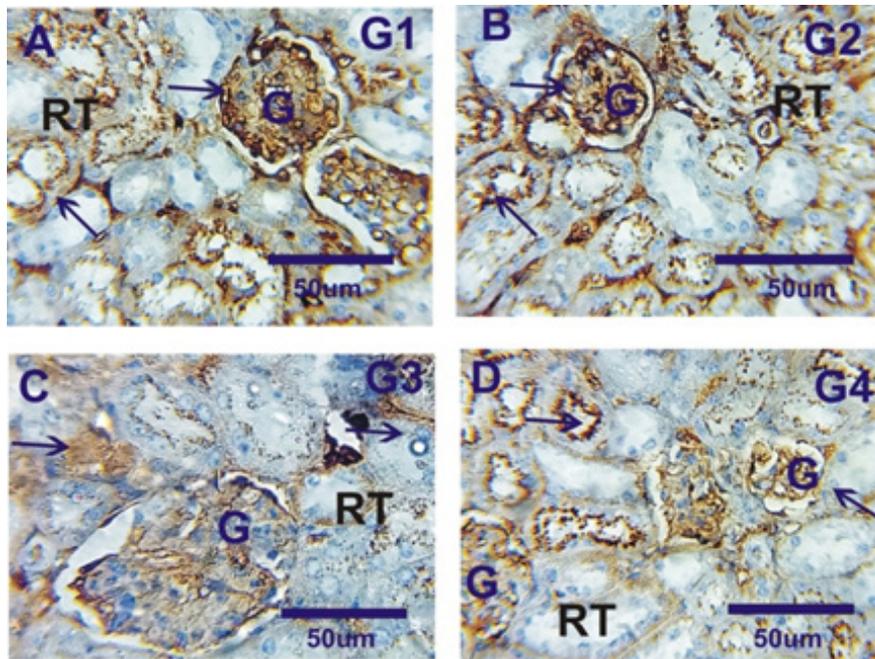


Fig. 3. Immunohistochemical localization of anti-apoptotic Bcl2 in kidney tissues in dissimilar groups. A&B: Strong positive responses for Bcl2 in kidney sections in control, and Poria NPs groups. C: Faint positive responses for Bcl2 at kidney slices in EST. D: Mild positive responses for Bcl2 at treated EST with Poria NPs group

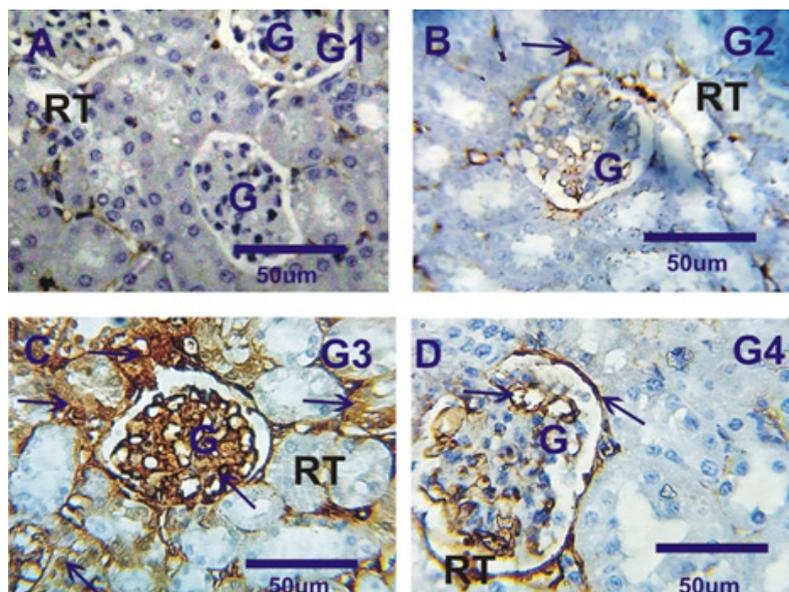


Fig. 4. Immunohistochemical localization of apoptotic P53 in kidney tissues in dissimilar groups. A&B: Faint positive responses for P53 in kidney sections in control, and Poria NPs groups. C: Strong positive responses for P532 at kidney slices in EST. D: Mild positive responses for P53 at treated EST with Poria NPs group

under study were demonstrated at figures (4&5). Kidney slices of control and Poria NPs show faint positive responses for P53 (grade 1) of glomeruli and renal tubules. Moderate to strong positive responses for P53 (grade 3), were detected in kidney slices in EST group. Moreover, mild positive responses for P53 (grade 2) were detected at treated EST with Poria NPs group.

DISCUSSION

Ehrlich carcinoma has equivalence with human cancers as it is undistinguished, has a speedy advancement average and more sensitive to chemotherapy.^{3,32} Tumour growth in an animal's body can affect a range of organ functions, and structure; therefore, current study aimed to study the impact of Poria NPs against EST induced kidney injury, toxicity, oxidative stress and apoptosis. Current results revealed that; EST induced defects in kidney function, which is established by increase the concentrations of creatinine, urea, chloride, potassium, and decrease in calcium and sodium levels, maybe attributable to EST induced toxicity and injury in kidney. In contrast; treatments of EST with Poria NPs improved these changes in kidney functions and alterations in electrolytes.

Increased urea and creatinine levels demonstrate lower glomerular filtration rates, indicating that the kidney is less efficient at excreting waste materials.³³ Furthermore, our findings were in line with Abd Eldaim *et al.*^{7,11}; Mutar *et al.*⁴ who reported that; EST causes renal impairment in mice through elevation in the levels of urea and creatinine.

Oxidative stress is tightly linked to every aspect of cancer, including tumor-bearing, treatment, and prevention. The tumor-bearing condition should be under oxidative stress brought on by the tumour cells actively producing oxygen and by faulty oxidation-reduction regulation.¹³ Our findings showed that in kidney homogenates from EST as compared to control, MDA levels were significantly higher while GSH and catalase activity were significantly lower. According to Tousson *et al.*⁵, Aldubayan *et al.*³, and El-Masry *et al.*⁸, EST caused an increase in MDA levels, which was accompanied by a decrease in GSH, SOD, and catalase levels in the blood and liver. Our findings corroborate their findings.

Our study found that inducing EST in mice changed the structure of kidneys as a result of the production of (ROS), which play a crucial role in oxidative stress by disrupting cellular

homeostasis and causing tissue destruction.^{3,33} Our biochemical outcomes were confirmed by the observations of histopathological and immunohistochemical investigation of Kidney, since Kidney section of EST bearing mice revealed renal toxicity manifested by significant deterioration and leanness of tubular cells and in glomeruli in kidney sections.

Apoptosis is an important process for keeping cell numbers in check, without affecting cell replication multiplication. P53 protein is a transcription factor responsible in cell cycle regulation, gene expression, ageing, apoptosis, and cancer suppression.¹⁶ In the current study, kidney sections in EST revealed significant increase in apoptotic P53 expressions while Bcl2 expressions were significantly decreased, indicating that EST induced apoptosis. Additionally, EST+Poria NPs adjust these changes in P53 and Bcl2 emphasizing that Poria NPs has anti-apoptotic effects. Our findings agree with Khalil *et al.*³⁴ who reported that *E. foeminea* extracts are a natural source of antioxidants that protect endothelium cells from free radical damage. Our findings revealed an unfavorable relationship amid p53 and Bcl-2 expressions. Our findings agree with Tousson *et al.*³¹ who reported the relation between p53 and Bcl-2 in cardiac tissues after treatments with amethopterin. Finally, the present work confirmed that the use of Poria NPs in treating mice bearing EST decreased P53 and increased Bcl2 expression of renal cells. Poria Nps is a potential adjuvant useful for the prevention and treatment of kidney toxicity and potentially applicable as a natural chemotherapeutic agent.

CONCLUSION

According to the findings of our study, the effects of Ehrlich solid tumors in mice were to induce kidney damage, oxidative stress, and modify the expression of p53 and Bcl2. Administering Poria NPs extract helped to kill the cancer cells through apoptosis which thereby regulates the proliferation of cancer cells and inhibits its spread to other organs. In comparison to chemotherapy, this novel medication has the ability to reduce the side effects significantly in normal cells in conjunction with complete spoilage for cancer cells.

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