OnLine Journal of Biological Sciences

Original Research Paper

Impact of B-Glucan Against Ehrlich Ascites Carcinoma Induced Renal Toxicity in Mice

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Article history
Received: 22-10-2022
Revised: 10-01-2023
Accepted: 13-01-2023

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Abstract: Several studies have been focused on identifying alternative medicines for the treatment of cancer. This study aimed to investigate the impact of B-Glucans extract (BG) against Ehrlich Ascites Carcinoma (EAC) induced toxicity and tissue damage in kidney. A total of 40 mice were assigned randomly to four groups (G1, control; G2, B-Glucans (75 mg/kg bw/day) orally for 2 weeks; G3, EAC and G4, EAC + BG). Current results revealed, EAC induced significant elevation in urea, creatinine, potassium (k^+), chloride (Cl^-) ions, kidney injury and significant decrease in sodium (Na^+) and calcium (Ca^{++}) ions when compared to B-glucans and control groups. Conversely, EAC + B-glucans modulate these changes and improved the renal functions and structure. EAC induced renal dysfunction and B-Glucans has the ability to improve renal functions. We can conclude that; BG could be used as an adjuvant therapy for kidney in ascites of hepatocellular carcinoma, *Schistosoma* and hepatitis.

Keywords: Ehrlich Ascites Carcinoma, B-Glucans, Kidney, Mice

Introduction

Cancer is an abnormal growth of cells caused by abnormal cell proliferation and can invade other parts of the body through circulation (Bray *et al*., 2018; Ferlay *et al*., 2019). According to World Health Organization (WHO), more than 10 million new cases of cancer are identified every year and it is the second leading cause of death all over the world (Siegel *et al*., 2022).

Breast cancer is a significant illness, with 1.41 million new diagnoses and 46,000 fatalities occurring each year (Oeffinger *et al*., 2015; La Rocca *et al*., 2020). Breast cancer has a high rate of morbidity and mortality and is the second leading cause of death in women (Pagani *et al*., 2010). Ehrlich Tumors that are Solid (EST) or in the form of ascites (EAC) induced various damage in most of body organs by creating oxidative stress (Aldubayan *et al*., 2019; El-Masry *et al*., 2019; 2020; Elgharabawy *et al*., 2021). For convenient research into the anti-cancer properties of several chemical compounds, the Ehrlich tumour (EST and EAC) has been adopted as a transplantable tumour model (Abd Eldaim *et al*., 2019; 2021a; Oshiba *et al*., 2021). Ehrlich Ascites Carcinoma (EAC) also known as an undifferentiated carcinoma, is initially hyperdiploid, highly transplantable, never regresses, proliferates quickly, has a shorter life span, and is completely malignant (Mutar *et al*., 2020; Alotaibi *et al*., 2021; Tousson *et al*., 2022).

Chemotherapy is the most widely used kind of cancer treatment that kill malignant growth cells by initiating apoptosis, but it has a major impact on patients' quality of life and is thought to be a direct cause of mortality (Tousson *et al*., 2014; 2016; 2018). Complementary and alternative medicine use has clearly increased in recent years (Saggu *et al*., 2014; Altwaijry *et al*., 2020; 2021; Mohamed *et al*., 2021).

B-Glucans (BG) are among these polysaccharides, that are found in the structural constituent of the cell walls of various micro-organisms including bacteria, yeast and fungi (Murphy *et al*., 2020; Rattanachan *et al*., 2020; Nguyen *et al*., 2022). BG is a natural product that is universally utilized in cosmetics, foods, and medicine (Gülmen, 2011; Choromanska *et al*., 2018; Dixon *et al*., 2022). In the medical field, BG are used to treat a many of diseases include diabetes, hypercholesterolemia, and HIV/AIDS (Choromanska *et al*., 2018). Therefore; current study aimed to study the impact of β-glucan on EAC induced changes in kidney functions, electrolytes and renal structure.

Materials and Methods

Transplantation EAC in Mice

Ehrlich Ascites Carcinoma (EAC) bearing mice were bought from the Egyptian National Cancer Institute. Ehrlich ascites carcinoma cells in mice (NCI; Cairo University, Egypt). Ehrlich ascites carcinoma (EAC) cells that are seven days old have been taken from mice that have the disease and suspended in sterile isotonic saline. Each mouse received an intraperitoneal injection of two and a half million live EAC cells to cause EAC (Abd Eldaim *et al*., 2021b).

Animals

40 mice (Swiss albino, female; 20-25 g) were obtained from Egyptian EVC animal house colony. Animals The creatures were randomly assigned to cages and kept in a room-temperature setting between 22 and 25°C with a 12 h light/12 h dark cycle, a commercial diet, and water for around 14 days.

The institutional animal care and use committee (IACUC-SCI-TU-00179) had the capability to underwrite this study which was guided by the rules provided by the Moral Committee of the Faculty of Science at Tanta University.

Experimental Design and Animal Groups

The four groups (Gp1-Gp4) of mice were distributed equally:

Gp1: Control Gp; where mice did not receive any therapy
Gp2: BG in which mice were received B-glucan orally (75 mg/kg bw/day) through a stomach tube for 14 days
Gp3: Ehrlich Ascites tumour Gp (EAC); mice were inoculated once intraperitoneal I.P with Ehrlich cells with approximately 2,500,000 EAC/mouse (Tousson *et al*., 2020)
Gp4: (EST + BG) Gp; mice were administered hypodermically with 2,500,000 EAC cells per mouse to induce tumour (EST) and 2nd days received B-glucan orally through stomach tube for 14 days

Blood and Tissue Sampling

After two weeks tumor inoculation, fluid cells of (EAC) were isolated from the peritoneal cavity of mice from infected group through withdrawing peritoneal fluid containing the tumor cells. In addition, all animals of each group were anaesthetized with intraperitoneal injection of sodium pentobarbital (≥100 mg/kg). Blood samples were collected by cardiac puncture and centrifuged at 3000 g for 20 min. Serum were separated and stored at -20°C until biochemical analysis. Kidney samples were removed, cleaned in cold saline and fixed in 10% neutral buffer formalin for histopathological investigations.

Electrolytes and Kidney Functions Biomarker

Serum urea and creatinine respectively were determined in the rat sera according to Oyouni *et al*. (2018); Abd Eldaim *et al*. (2019) respectively. Estimation of the levels of serum electrolytes (Potassium, sodium, calcium and chloride ion) by using commercial kits (Sensa core electrolyte, India) according to Elmasry *et al.* (2018).

Histological Preparation

After necropsy the kidney was immediately removed and fixed by immersion in 10% neutral buffered formalin solution for 24-48 h. The specimens were then dehydrated, cleared and embedded in paraffin. Serial sections of 5 µm thick were cut by mean of rotary microtome (Litz, Wetzlar; Germany) and stained with haematoxylin and eosin (Tousson, 2016).

Statistical Analysis

An unpaired t-test to evaluate the salient variances among treatment groups enabled the undertaking of statistical analysis where data were expressed as average values ± SE.

0.05 served as the threshold for the biochemical data representing the criterion of statistically significant data. The SPSS statistical version 21 software packages (SPSS® Inc., USA) were used to carry out all statistical analyses.

Results

Changes in Kidney Functions and Electrolytes

Table (1) showed that; EAC induced significant elevation in the levels of serum urea, creatinine, K^+, Cl^- and significant decrease in Na^+ and Ca^{++} when compared to B-glucan and control groups. On the other hand; treatments of EAC with B-glucan (EAC + BG) modulate these changes in blood parameters where a depletion in the levels of serum urea, creatinine, K^+, Cl^- and significant elevation in Na^+ and Ca^{++} when compared with EAC (Table 1).

Kidney Histopathology

Figure (1) showed kidney sections in control (Gp1) and B-glucan (Gp2) groups revealed normal histological structures of the glomeruli and renal tubules in the cortical and medullary portions (Fig. 1A-B).

Variable histopathological changes as moderate atrophied glomeruli and marked degenerated and atrophy of tubular cells with moderate inflammatory cellular infiltration on kidney sections in EST (Gp3) group (Fig. 1C). In contrast; treatments of EST with B-glucan (Gp4; EAC + BG) induced minimal histopathological changes in kidney as mild atrophy in glomeruli and tubular cells (Fig. 1D).

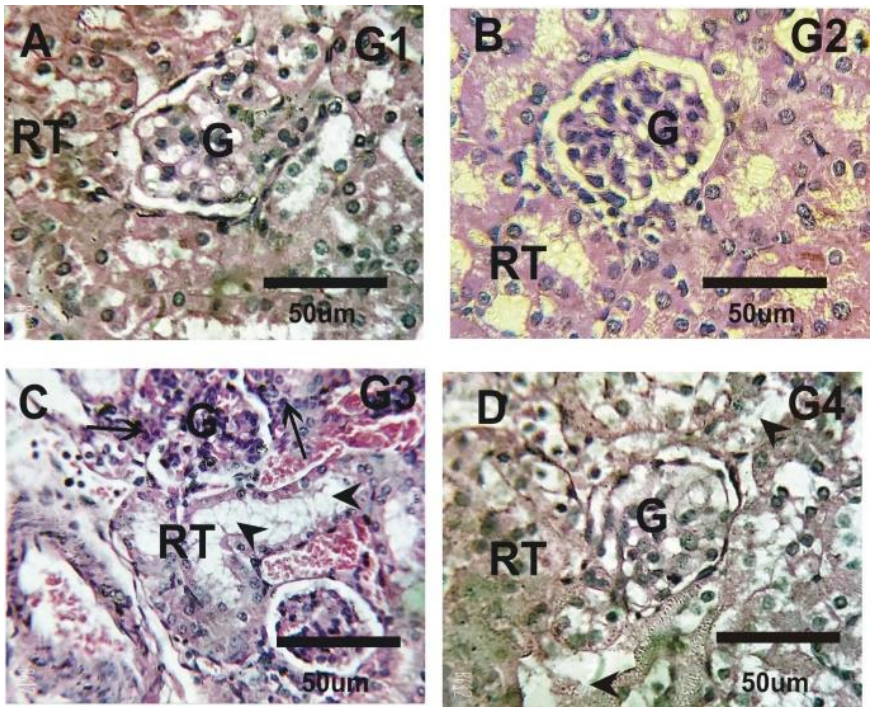

Fig. 1: Kidney T's photomicrographs stained with H and E in different groups. (A and B): Normal histological structures of the Glomeruli (G) and renal Tubules (Tb) in control (G1) and B-Glucan (G2) groups respectively. (C): Moderate atrophied glomeruli and marked degenerated and atrophy of tubular cells with moderate inflammatory cellular infiltration on kidney sections in EST (black arrows) (white arrows). (D): Mild atrophy in glomeruli and tubular cells (arrowheads) in the kidney in EST + B-glucan

Table 1: Changes in levels of kidney functions and electrolytes in different groups

	Control	BG	EAC	EAC + BG
Urea (mg/dl)	$35.4^{\#}\pm1.89$	$34.0^{\#}1.45$	$48.5^{*}\pm1.90$	$37.6^{\#}\pm2.05$
Creatinine (mg/dl)	$0.51^{\#}\pm0.02$	$0.47^{\#}\pm0.02$	$1.18^{*}\pm0.08$	$0.64^{\#*}\pm0.04$
Na^{+} (mmol/l)	$135.4^{\#}\pm8002E8$	$135.8^{\#}\pm9.6$	$143.0^{*}\pm10.5$	$137.7^{\#}\pm10.6$
K^{+} (mmol/l)	$4.22^{\#}\pm0.15$	$4.40^{\#}\pm0.28$	$6.36^{*}\pm0.35$	$5.35^{\#*}\pm0.31$
Ca^{++} (mmol/l)	$1.12^{\#}\pm0.06$	$1.18^{\#}\pm0.08$	$0.763^{*}\pm0.02$	$0.922^{\#*}\pm0.04$
Cl^{-} (mmol/l)	$101.6^{\#}\pm8.3$	$102.5^{\#}\pm9.0$	$117.5^{*}\pm8.2$	$118.5^{\#*}\pm9.1$

Data are expressed as mean ± SE of 10 observations. Significant difference from the control group at * $p\leq0.05$. Significant difference from EAC group at # $p\leq0.05$

Discussion

The anticancer capabilities of BG have garnered a lot of interest recently across the world, although more clinical study and experimental work are still required. Therefore, the current research aimed to examine the therapeutic potential of BG extract in treating kidney toxicity triggered by EAC. Our outcomes indicate that EAC initiate changes in kidney functions as detected by elevation in levels of urea, creatinine, potassium, and chloride ions and depletion in levels of sodium and calcium ions.

This variation in kidney functions may be connected to renal tissue injury brought on by EAC. Also; current results revealed that EAC induced kidney tissues damage as marked atrophy in glomeruli and tubular cells in addition to marked degenerated tubular cells. These results corroborated those of Abd Eldaim *et al*. (2021a); Mutar *et al*. (2020) who found that; EAC induced elevation in kidney functions and tissue injury.

These results concurred with those of Abd Eldaim *et al*. (2019); Aldubayan *et al*. (2019); Tousson *et al*. (2022) who reported that; EST induced kidney toxicity in female mice. Current results revealed that; treatment of EST with star anise extract improved kidney functions. These outcomes were consistent with reports of EAC-induced liver damage from Abd Eldaim *et al*. (2021b); Aldubayan *et al*. (2019); Tousson *et al*. (2020).

The fact that histopathological analysis of the kidney tissues of EAC-bearing mice revealed renal toxicity and correlated with Mutar *et al*. (2020) who's findings that EAC caused kidney injuries and the destruction of hepatocytes, which released their enzymes into the plasma, suggests that these increases in liver enzyme

activities may be the result of hepatic tissue injuries. In additionally (Salem *et al*., 2011) indicated that EAC induced degenerated in the renal tubules and atrophy in the glomerulus as well as proteinaceous casts and leucocyte infiltration in the lumen of the renal tubules.

Our results correspond with Bashir and Choi (2017); Dawood *et al*. (2020) who reported that; B-glucan improved the kidney functions where it increases the decrease in creatinine and urea levels in chronic kidney disease and it may reflect increased gut permeability and altered mononuclear phagocytic system function.

Also with Vetvickova (2020) who studies the effect of Oat β-Glucan Supplementation on Chronic Kidney Disease. Also, with Wang *et al*. (2020) who reported that; B-glucan ameliorates renal function and gut microbiota in diabetic rats.

Conclusion

EAC induced renal dysfunction where it elevates the levels of urea, creatinine, potassium and chloride in addition to induced renal damage and B-glucan has the ability to improve renal functions. These findings suggest that B-glucan can be a reliable and novel therapy for cancer, further validate the neoplastic activity of BG as a potential therapy for other types of cancer is needed. We can conclude that; B-glucan could be used as an adjuvant therapy in ascites of HCC, *Schistosoma* and hepatitis.

Acknowledgment

This study did not receive any grant from any funding agency.

Declaration of Conflicting Interests

The author's declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data Availability

Data supporting our study results are accessible from the relevant author whenever needed.

Funding Information

The author's received no financial support for the research, authorship, and/or publication of this article.

Author's Contributions

Ahmed F. Hasan and Alaa Saadi Abbood: Wrote the first draft of the manuscript.

Hiba Mohammed Al-Khuzaay and Azhar Azher Alankooshi: Performed the statistical analysis and approved the final manuscript.

Ali Ghazi Dulimi and Eslam Ahmed Elsaedy: Managed the analyses of the study and managed the literature searches.

Ehab Tousson: Designed the study and wrote the protocol, read and approved the final manuscript.

Ethics

The study design was endorsed by the Institutional Ethical Committee for Animal Care and Use (code: IACUC-SCI-TU-0179).

References

Abd Eldaim, M. A., Tousson, E., El Sayed, I. E. T., Abd El, A. E. A. H., & Elsharkawy, H. N. (2019). Grape seeds proanthocyanidin extract ameliorates Ehrlich solid tumor-induced renal tissue and DNA damage in mice. *Biomedicine & Pharmacotherapy*, *115*, 108908. https://doi.org/10.1016/j.biopha.2019.108908

Abd Eldaim, M. A., Tousson, E., El Sayed, I. E. T., Abd Elmaksoud, A. Z., & Ahmed, A. A. (2021a). Ameliorative effects of 9-diaminoacridine derivative against Ehrlich ascites carcinoma-induced hepatorenal injury in mice. *Environmental Science and Pollution Research*, *28*, 21835-21850.

Abd Eldaim, M. A., Tousson, E., Soliman, M. M., El Sayed, I. E. T., Abdel Aleem, A. A. H., & Elsharkawy, H. N. (2021b). Grape seed extract ameliorated Ehrlich solid tumor-induced hepatic tissue and DNA damage with reduction of PCNA and P53 protein expression in mice. *Environmental Science and Pollution Research*, *28*, 44226-44238. https://doi.org/10.1007/s11356-021-13904-8

Aldubayan, M. A., Elgharabawy, R. M., Ahmed, A. S., & Tousson, E. (2019). Antineoplastic activity and curative role of avenanthramides against the growth of ehrlich solid tumors in mice. *Oxidative Medicine and Cellular Longevity*, *2019*. https://doi.org/10.1155/2019/5162687

Alotaibi, B., Tousson, E., El-Masry, T. A., Altwaijry, N., & Saleh, A. (2021). Ehrlich ascites carcinoma as model for studying the cardiac protective effects of curcumin nanoparticles against cardiac damage in female mice. *Environmental Toxicology*, *36*(1), 105-113. https://doi.org/10.1002/tox.23016

Altwaijry, N., El-Masry, T. A., Alotaibi, B., Tousson, E., & Saleh, A. (2020). Therapeutic effects of rocket seeds (*Eruca sativa* L.) against testicular toxicity and oxidative stress caused by silver nanoparticles injection in rats. *Environmental Toxicology*, *35*(9), 952-960. https://doi.org/10.1002/tox.22931

Altwaijry, N., El-Masry, T. A., Alotaibi, B. S., Tousson, E., Alodhayani, A. A., El-Morshedy, K., ... & Saleh, A. (2021). Potential therapeutic effects of avenanthramide-C against lung toxicity caused by silver nanoparticles injection in rats. *Pakistan Journal of Pharmaceutical Sciences*, *34*. https://doi.org/10.36721/PJPS.2021.34.1.SUP.337-343.1

Bashir, K. M. I., & Choi, J. S. (2017). Clinical and physiological perspectives of β-glucans: The past, present and future. *International Journal of Molecular Sciences*, *18*(9), 1906. https://doi.org/10.3390/ijms18091906

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394-424. https://doi.org/10.3322/caac.21492

Choromanska, A., Kulbacka, J., Harasym, J., Oledzki, R., Szewczyk, A., & Saczko, J. (2018). High-and low-molecular weight oat beta-glucan reveals antitumor activity in human epithelial lung cancer. *Pathology & Oncology Research*, *24*, 583-592. https://doi.org/10.1007/s12253-017-0278-3

Dawood, M. A., Abdel-Razik, N. I., Gewaily, M. S., Sewilam, H., Paray, B. A., Soliman, A. A., ... & El Basuini, M. F. (2020). β-Glucan improved the immunity, hepato-renal and histopathology disorders induced by chlorpyrifos in Nile tilapia. *Aquaculture Reports*, *18*, 100549. https://doi.org/10.1016/j.aqrep.2020.100549

Dixon, B., Hoseinifar, S. H., Huyben, D., Kestemont, P., & Khuyen, T. D. (2022). GAS1: A New b-Glucan Immunostimulant Candidate to Increase Rainbow Trout. *Oral Immune-Enhancing Research in Fish*.

Elgharabawy, R. M., El Tantawy El Sayed, I., Abd-Allah Rezk, N., & Tousson, E. (2021). Therapeutic impact of costus (*Saussurea lappa*) against Ehrlich solid tumor-induced cardiac toxicity and DNA damage in female mice. *Frontiers in Pharmacology*, *12*, 708785. https://doi.org/10.3389/fphar.2021.708785

El-Masry, T., Al-Shaalan, N., Tousson, E., Buabeid, M., & Al-Ghadeer, A. (2020). Potential therapy of vitamin B17 against Ehrlich solid tumor induced changes in Interferon gamma, Nuclear factor kappa B, DNA fragmentation, p53, Bcl2, survivin, VEGF and TNF-α Expressions in mice. *Pakistan Journal of Pharmaceutical Sciences*, *33*. https://doi.org/10.36721/PJPS.2020.33.1.SUP.393-401.1

El-Masry, T. A., Al-Shaalan, N. H., Tousson, E., Buabeid, M., & Alyousef, A. M. (2019). The therapeutic and antineoplastic effects of vitamin B17 against the growth of solid-form Ehrlich tumours and the associated changes in oxidative stress, DNA damage, apoptosis and proliferation in mice. *Pak. J. Pharm. Sci*, *32*(6), 2801-2810. https://www.researchgate.net/publication/337935002

Elmasry, T. A., Al-Shaalan, N. H., Tousson, E., El-Morshedy, K., & Al-Ghadeer, A. (2018). Star anise extracts modulation of reproductive parameters, fertility potential and DNA fragmentation induced by growth promoter Equigan in rat testes. *Brazilian Journal of Pharmaceutical Sciences*, *54*. https://doi.org/10.1590/s2175-97902018000117261

Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D. M., Piñeros, M., ... & Bray, F. (2019). Estimating the global cancer incidence and mortality in 2018: Globocan sources and methods. *International Journal of Cancer*, *144*(8), 1941-1953. https://doi.org/10.1002/ijc.31937

Gülmen, Ş. (2011). The effect of beta-glucan on kidney injury in experimental aortic ischemia-reperfusion. *Turkish Journal of Thoracic and Cardiovascular Surgery*, *19*(2). https://tgkdc.dergisi.org/uploads/pdf/pdf_TGKDC_1389.pdf

La Rocca, E., Meneghini, E., Lozza, L., Fiorentino, A., Vitullo, A., Giandini, C., ... & De Santis, M. C. (2020). Older age and comorbidity in breast cancer: is RT alone the new therapeutic frontier? *Journal of Cancer Research and Clinical Oncology*, *146*, 1791-1800. https://doi.org/10.1007/s00432-020-03243-5

Mohamed, A. E., El-Magd, M. A., El-Said, K. S., El-Sharnouby, M., Tousson, E. M., & Salama, A. F. (2021). Potential therapeutic effect of thymoquinone and/or bee pollen on fluvastatin-induced hepatitis in rats. *Scientific Reports*, *11*(1), 1-12. https://doi.org/10.1038/s41598-021-95342-7

Murphy, E. J., Masterson, C., Rezoagli, E., O'Toole, D., Major, I., Stack, G. D., ... & Rowan, N. J. (2020). β-Glucan extracts from the same edible shiitake mushroom Lentinus edodes produce differential in-vitro immunomodulatory and pulmonary cytoprotective effects-Implications for coronavirus disease (COVID-19) immunotherapies. *Science of the Total Environment*, *732*, 139330. https://doi.org/10.1016/j.scitotenv.2020.139330

Mutar, T. F., Tousson, E., Hafez, E., Abo Gazia, M., & Salem, S. B. (2020). Ameliorative effects of vitamin B17 on the kidney against Ehrlich ascites carcinoma induced renal toxicity in mice. *Environmental Toxicology*, *35*(4), 528-537. https://doi.org/10.1002/tox.22888

Nguyen, D. T., Marancik, D., & Soto, E. (2022). B-glucan immunostilulation against columnaris in a white sturgeon (*Acipenser transmontanus*) model. *Fish and Shellfish Immunology Reports*, *3*, 100067. https://doi.org/10.1016/j.fsirep.2022.100067

Oeffinger, K. C., Fontham, E. T., Etzioni, R., Herzig, A., Michaelson, J. S., Shih, Y. C. T., ... & Wender, R. (2015). Breast cancer screening for women at average risk: 2015 guideline update from the American Cancer Society. *Jama*, *314*(15), 1599-1614. https://doi.org/10.1001/jama.2015.12783

Oshiba, R. T., Touson, E., Elsherbini, Y. M., & Abdraboh, M. E. (2021). Melatonin: A regulator of the interplay between FoxO1, miR96 and miR215 signaling to diminish the growth, survival and metastasis of murine adenocarcinoma. *BioFactors*, *47*(5), 740-753. https://doi.org/10.1002/biof.1758.

Oyouni, A. A. A., Saggu, S., Tousson, E., & Rehman, H. (2018). Immunosuppressant drug tacrolimus induced mitochondrial nephrotoxicity, modified PCNA and Bcl-2 expression attenuated by *Ocimum basilicum* L. in CD1 mice. *Toxicology Reports*, *5*, 687-694. https://doi.org/10.1016/j.toxrep.2018.06.003

Pagani, O., Senkus, E., Wood, W., Colleoni, M., Cufer, T., Kyriakides, S., ... & Cardoso, F. (2010). International guidelines for management of metastatic breast cancer: Can metastatic breast cancer be cured? *Journal of the National Cancer Institute*, *102*(7), 456-463. https://doi.org/10.1093/jnci/djq029

Rattanachan, S., Bunnajirakul, S., & Punyadarsaniya, D. (2020). Effect of pre-supplementation with Pleurotus sajor-caju crude extracts on body weight and consequence responses of leukocytes and immune organs in fancy carp following inoculation with Aeromonas veronii. *Veterinary World*, *13*(5), 1010. https://doi.org/10.14202/vetworld.2020.1010-1016

Saggu, S., Sakeran, M. I., Zidan, N., Tousson, E., Mohan, A., & Rehman, H. (2014). Ameliorating effect of chicory (*Chichorium intybus* L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. *Food and Chemical Toxicology*, *72*, 138-146. https://doi.org/10.1016/j.fct.2014.06.029

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2022). Cancer statistics, 2022. *CA: A Cancer Journal for Clinicians*, *72*(1), 7-33. https://doi.org/10.3322/caac.21708

Tousson, E., Bayomy, M. F., & Ahmed, A. A. (2018). Rosemary extract modulates fertility potential, DNA fragmentation, injury, KI67 and P53 alterations induced by etoposide in rat testes. *Biomedicine & Pharmacotherapy*, *98*, 769-774. https://doi.org/10.1016/j.biopha.2018.01.025

Tousson, E., Hafez, E., Abo Gazia, M. M., Salem, S. B., & Mutar, T. F. (2020). Hepatic ameliorative role of vitamin B17 against Ehrlich ascites carcinoma-induced liver toxicity. *Environmental Science and Pollution Research*, *27*, 9236-9246. https://doi.org/10.1007/s11356-019-06528-6

Tousson, E., El Sayed, I. E., Abd El-Aleim, H., Elabd, M., Karhib, M., & Gebreel, D. T. (2022). Impact of Poria Cocos Nanoparticles Extract Against Ehrlich Solid Tumour Induced Toxicity, Oxidative Stress and Apoptosis in Female Mice Kidney. *Biomedical and Pharmacology Journal*, *15*(4), 1927-1935. https://doi.org/10.13005/bpj/2531

Tousson, E., Hafez, E., Zaki, S., & Gad, A. (2016). The cardioprotective effects of L-carnitine on rat cardiac injury, apoptosis and oxidative stress caused by amethopterin. *Environmental Science and Pollution Research*, *23*, 20600-20608. https://doi.org/10.1007/s11356-016-7220-1

Tousson, E., Hafez, E., Zaki, S., & Gad, A. (2014). P53, Bcl-2 and CD68 expression in response to amethopterin-induced lung injury and ameliorating role of l-carnitine. *Biomedicine & Pharmacotherapy*, *68*(5), 631-639. https://doi.org/10.1016/j.biopha.2014.05.007

Tousson, E. (2016). Histopathological alterations after a growth promoter boldenone injection in rabbits. *Toxicology and Industrial Health*, *32*(2), 299-305. https://doi.org/10.1177/0748233713500821

Vetvickova, J. (2020). Urinary tract infection-evaluation of Beta-glucan, mannose and cranberry extract. *World Journal of Pathology*, *9*(3). https://www.npplweb.com/wjp/fulltext/9/3

Wang, N., Liu, H., Liu, G., Li, M., He, X., Yin, C., ... & Yin, H. (2020). Yeast β-D-glucan exerts antitumour activity in liver cancer through impairing autophagy and lysosomal function, promoting reactive oxygen species production and apoptosis. *Redox biology*, *32*, 101495. https://doi.org/10.1016/j.redox.2020.101495