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Roles of Chitosan Nanoparticles on Ehrlich Ascites Carcinoma Induced Changes in Some Blood Parameters

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ET and DTG designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SA managed managed the literature searches and the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objective: Chitosan, the deacetylated derivative of chitin, is a natural D-glucosamine polymer that can be extracted from the shells of seafood such as lobsters and prawns crabs which have obtained a great interest in cancer therapy. This study aimed to investigate the hepatic and renal protective potential of chitosan nanoparticles (CNPs) against Ehrlich ascites carcinoma (EAC)–bearing mice- induced blood toxicity.

Materials and Methods: A total of 40 female mice were divided into 4 groups (1st group, control group; 2nd group, CNPs group; 3rd group, EAC group; 4th group, co-treated EAC with CNPs).

Results: Results showed that the presence of CNPs in co-treated groups lead to decreased cholesterol, triglyceride, urea, creatinine, AST, ALT, ALP, potassium and chloride ions levels while

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showed increased total protein, albumin, sodium and calcium ions levels compared to EAC group.

Conclusion: The present study confirmed that chitosan nnp has a protective potential effect against EAC cell induced blood toxicity toxicity.

Keywords: Chitosan NPs; EAC; mice; blood; electrolyte; toxicity.

1. INTRODUCTION

For years, cancer has been one of the biggest threats to human life; it is expected to become the leading cause of death over the next few decades [1]. Based on statistics from the World Health Organization (WHO), cancer accounted for 13% of all deaths in the world in 2004; deaths caused by cancer are expected to increase in the future, with an estimated 12 million people dying from cancer in 2030 [1, 2, 3]. There are a number of in vivo experimental models based on laboratory animals including the Ehrlich solid tumor, derived from the mouse breast adenocarcinoma, which is an aggressive and fast growing carcinoma able to develop both in the ascitic and the solid form depending on whether inoculated intra-peritoneal or subcutaneously, respectively [4,5]. Therefore, the solid and the ascitic forms of this tumor are frequently utilized to evaluate the tumor pathogenesis and development of antitumor activity of different products [6, 7, 8, 9].

Ehrlich ascites carcinoma (EAC) is one of the commonest tumors. EAC is referred to as an undifferentiated carcinoma and is originally hyper diploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen (TSTA) [7, 10, 11, 12]. EAC resembles human tumors which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth rate [10, 13, 14]. Frequently, tumor virulence increases via repetitious passages, while the proliferating rate of such tumors increases gradually. However, the differentiation gradually disappears, while the cells get free growth control mechanisms, gain hetero-transplantability and in the end, they are converted to the ascites' form [10, 15].

According to numerous studies, chitosan was used in anticancer, anti-inflammation, reducing renal and hepatotoxicity, and treat many diseases [16]. Chitosan, also known as β (1-4)-linked 2-acetamido-2-deoxy-β-D-glucose (N-acetyl glucosamine), is a cationic polysaccharide investigated as a potential adjuvant in human vaccines due to its biocompatible and biodegradable nature. It is derived via the chemical de-acetylation of chitin, one of the most abundant polymers in nature, second only to cellulose, which is found naturally in the exoskeleton of crustacean, insects, and some fungi [17, 18]. Several studies supply positive evidence to protective effects of chitosan and its bioactive components as well as the mechanism of action against some tumors and diseases. Chitosan can inhibit the proliferation of hepatic cancer, bladder cancer, ovarian cancer, Crohn disease and High blood pressure [19, 20, 21]. This study aimed to study the effect of chitosan nanoparticles (NPs) on Ehrlich ascites carcinoma (EAC) induced variations in liver and kidney functions, electrolytes and lipid profiles.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty female Swiss albino mice were used in the present study. Mice were divided into four equal groups, ten mice in each group: Group1, include mice were not injected with anything kept as a control; Group 2, include animals inoculated with chitosan nanoparticles for two weeks; Group 3, include animals inoculated once intra-peritoneal (I.P) with Ehrlich cells and Group 4, is co-treated group where animals inoculated once intra-peritoneal (I.P) with Ehrlich cells and treated at the same time with chitosan NPs for two weeks.

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 diethyl ether and sacrificed. Blood samples and liver and kidney tissues were immediately removed for biochemical, histological studies. Animals were obtained from animal house colony Egypt Vaccine Company, Giza, Egypt, as approved by Institutional Animal Care and Use Committee (IACUC-SCI-TU-0166).

2.2 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 allowed to stand for 30 min at 4oC for clot formation and centrifuged for 10 minutes at 3000 rpm. The collected serum was stored at -18° C until analysis for estimation of some blood parameters.

2.2.1 Liver functions test

The activities of serum aspartate aminotransferase (AST) and alanine transaminase (ALT) according to Moustafa et al. [22], and Alrasheed et al. [23] respectively.

While serum levels of alkaline phosphatase (ALP) was estimated according to El Moghazy et al. [24]. Serum levels of total protein were determined by using commercial kit according to Tousson et al. [25]. Serum levels of albumin were determined by using commercial kit according to [26].

2.2.2 Kidney functions and electrolytes

Serum urea and creatinine were determined in the mouse sera according to El Moghazy et al. [24]. Serum potassium, sodium, calcium and chloride ions levels in was determined by using commercial kits (Sensa core electrolyte, India) according to Basuony et al. [26].

2.2.3 Measurement of lipid profiles

Cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels were determined with Kits from ELLTECH, according to El Atrash et al. [27].

2.3 Statistical Analysis

Data were expressed as mean values ± SE and statistical analysis was performed using unpaired t-test to assess significant differences among treatment groups. The criterion for statistical significance was set at $p<0.05$ for the biochemical data. All statistical analyses were performed using SPSS statistical version 21 software package (SPSS® Inc., USA).

3 RESULTS

3.1 Biochemical Investigations

3.1.1 Liver and kidney functions parameters of EAC bearing mice

Table 1 and 2 represented the data of the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, urea and creatinine in serum of Ehrlich ascites carcinoma (EAC) bearing female mice. The results showed significantly ($P<0.0001$) increased in the activity of liver enzymes (AST, ALT and ALP) and kidney enzymes (urea and creatinine) and a significant ($P<0.0001$) decrease in the serum total protein and albumin in (EAC) bearing mice group compared with normal control and co-treated groups.

Also, Table 3 showed that; a significant of EAC group increase in levels of serum cholesterol at ($p<0.0001$), serum triglyceride at ($P<0.0001$), serum LDL at ($p<0.0001$) and a significant decrease in levels of serum HDL at ($p<0.0001$) compared with control and chitosan NPs groups (Table 2). Table 4 showed that; serum potassium and chloride levels were significantly increase and decrease in sodium and calcium levels in EAC group when compared with control and chitosan NPs groups.

4. DISCUSSION

Several biomolecules have shown many biological activities for immunomodulatory and anti-tumor activities [28]. The present work is

Table 1. Changes in serum liver enzymes (AST, ALT, ALP, total protein, albumin) levels in experimental groups

	Control	Chitosan NPs	EAC	Co-treated
AST (U/L)	132.5±4.77****	131.3± 4.61****	246.5 ± 9.31	158.5± 4.27****
ALT (U/L)	44 ± 1.47****	46.6 ± 1.05****	83.75 ± 2.87	54.7 ± 1.29****
ALP (U/L)	129 ± 2.68****	117.3± 3.33****	184.3 ± 5.07	147.8±3.198****
Total protein (g/l)	6.04±0.042****	6.22± 0.093****	5.25 ± 0.061	5.95±0.055****
Albumin (g/l)	4.41±0.031****	4.84± 0.052****	3.42±0.089	4.08 ± 0.052****

The significance of difference was analyzed by one – way ANOVA (compare all vs. EAC group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at P < 0.05

Table 2. Changes in serum kidney enzymes (urea and creatinine) levels in experimental groups

	Control	Chitosan NPs	EAC	Co-treated
Urea (mg/dl)	27.13±0.966****	34.13±1.197****	54.13± 3.0	39.13±0.826****
Creatinine(mg/dl)	0.55 ± 0.018****	0.47±0.034****	0.90±0.067	0.52 ± 0.029***

The significance of difference was analyzed by one – way ANOVA (compare all vs. EAC group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at P < 0.05

Table 3. Changes in serum lipid profiles (cholesterol, Tg, HDL, LDL) levels in experimental groups

	Control	Chitosan NPs	EAC	Co-treated
Cholesterol(mg/dl)	102.3±2.333****	108.7±2.404****	237.3±7.126	143±3.606****
Tg (mg/dl)	175.3±3.844****	184.3±7.055****	406 ± 16.04	248.7±5.487****
HDL(mg/dl)	36.97±1.633****	37.13±0.593****	26.83 ±0.601	44.67±1.167****
LDL (mg/dl)	30.3 ± 1.212****	34.67 ±0.968****	129.3 ±3.879	48.6 ± 3.703****

The significance of difference was analyzed by one – way ANOVA (compare all vs. EAC group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at P < 0.05

Table 4. Changes in serum sodium ions (Na+), potassium ions (K+), calcium ions (Ca2+) and chloride (Cl-) levels in experimental groups

	Control group	Chitosan Nps group	EAC Group	Co-treated group
Na^+ (mEq/L)	136.5±0.533****	135.3±0.629****	120± 1.646	133.1±0.898****
K^+ (mEq/L)	4.313±0.033****	4.33±0.025****	5.72±0.127	4.26± 0.102****
Cl^- (mEq/L)	100.9±0.850****	101.6±0.718****	116.1±1.45	103.8±0.160****
Ca^{2+} (mEq/L)	1.12 ± 0.003****	1.21±0.006****	0.912±0.013	1.09 ±0.016****

The significance of difference was analyzed by one – way ANOVA (compare all vs. EAC group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at P < 0.05

aimed to study the anti-neoplastic activity of chitosan NPs in EAC tumor bearing mice. The data obtained from this study revealed that chitosan NPs treated animals at inhibited the tumor volume, packed cell volume, tumor cell count (viable and nonviable) and reverted back the hematological parameters to more or less normal levels. In EAC tumor bearing mice, a regular rapid increase in ascites fluid volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [29].

In this study, affected liver, cause's elevated levels of enzymes due to liver cells' spills of enzymes in the blood, which indicates liver damage such as liver necrosis and inflammation, also, the high levels of kidney enzymes due to kidney fibrosis according to El-Wahab et al. [30]. Tousson et al. [12] who explained the increase in AST, ALT and ALP levels may indicate to liver tumor, liver necrosis, hepatitis, acute renal failure, acute pancreatitis, primary muscle disease and progressive muscular dystrophy. Our data revealed that the modified EAC kidney induction, which is indicated by increasing serum levels in urea, creatinine, potassium and chloride ions and low serum level of sodium and calcium ions that may be caused by the EAC that resulted in injury to the renal tissue. These findings were in line with that of Adwas et al. [31] who reported that tumor increased serum creatinine level in mice.

In addition, [12] demonstrated that EAC increases serum levels of urea and creatinine in female mice. These findings confirmed those of El-Wahab et al. [30], Badr et al. [32] who reported that EAC induced glomerulus atrophy, degenerative renal tubules, leucocyte infiltration and proteinaceous casts in the lumen of the renal tubules. Regarding the protective and treatment effects of chitosan NPs against EAC induced hebetic and renal damages, our results revealed that chitosan NPs modulated the effects of EAC on kidney and liver functions and structures, which is indicated by normalization of serum levels of liver and kidney enzymes and also serum of potassium, chloride, sodium and calcium. These results matched with that of Kim et al. [33] who indicated that chitosan NPs has protective effect against EAC induced acute

kidney and liver injury. In addition, Adhikari et al. [34] reported that chitosan NPs modulates induced alteration of serum levels of some electrolytes such as sodium, potassium, calcium and chloride ions in bearing mice of EAC.

Also, in the current study there was a significant increase in cholesterol, triglycerides and LDL and lower HDL levels in the EAC group as opposed to chitosan NPs group. Salem et al. [35] and El-Masry et al. [9] who confirmed that chitosan nanoparticles improves levels of lipid profiles caused by EAC. Also, our results revealed that EAC induced many remarkable degenerative changes by disorganization of the hepatic cords, in addition to karyomegaly and pyknotic nuclei indicating apoptosis, moderate fibrosis, and marked diffuse necrosis of hepatic tissue, marked inflammatory cells, and congested blood sinusoids. Our results agree with Badr et al. [36], and Tousson et al. [12].

5. CONCLUSION

Our results indicated that EAC altered serum levels of liver and kidney function biomarkers and some electrolytes and renal tissue structure. However, treatment of EAC bearing mice with chitosan NPs normalized serum levels of the altered parameters and ameliorated the effects of EAC on liver and kidney structure which chitosan NPs had potential protective effects against EAC induced liver and kidney injury.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bray F, Jemal A, Grey N, Ferlay J, Forman D. Global cancer transitions according to the human development index (2008–2030): A population-based study. The Lancet Oncology. 2012;13(8):790-801.
2. Gomes B, Higginson IJ. Where people die (1974—2030): Past trends, future projections and implications for care. Palliative Medicine. 2008;22(1):33-41.
3. Zali H, Rezaei-Tavirani M, Azodi M. Gastric cancer: Prevention, risk factors and treatment. Gastroenterology and Hepatology from Bed to Bench. 2011;4(4):175.
4. Abd Eldaim MA, Tousson E, El Sayed IET, Abd El AEAH, Elsharkawy HN. Grape seeds proanthocyanidin extract ameliorates ehrlich solid tumor induced renal tissue and dna damage in mice. Biomedicine & Pharmacotherapy. 2019; 115:108908.
5. El-Atrsh A, Tousson E, Elnahas EE, Massoud A, Al-Zubaidi M. Ameliorative effects of spirulina and chamomile aqueous extract against mice bearing ehrlich solid tumor induced apoptosis. Asian Oncology Research Journal. 2019;1-17.
6. Abd Eldaim MA, Tousson E, El Sayed IE, Awd WM. Ameliorative effects of saussurea lappa root aqueous extract against ethephon-induced reproductive toxicity in male rats. Environmental Toxicology. 2019;34(2):150-9.
7. Ahmed M, Ehab T, Ahmed SNE, Mona EM, Haneen HM. Antineoplastic activities of grape seed proanthocyanidin extract against ehrlich solid tumor bearing mice induced alterations in afp, cea, tnf-α and dna damage. Asian Oncology Research Journal. 2019;1-12.
8. Aldubayan MA, Elgharabawy RM, Ahmed AS, Tousson E. Antineoplastic activity and curative role of avenanthramides against the growth of ehrlich solid tumors in mice. Oxid. Med. Cell. Longev. 2019.
9. El-Masry T, Al-Shaalan N, Tousson E, Buabeid M, Al-Ghadeer A. Potential therapy of vitamin b17 against ehrlich solid tumor induced changes in interferon

gamma, nuclear factor kappa b, dna fragmentation, bcl2, survivin, vegf and tnf-α expressions in mice. Pak. J. Pharm. Sci. 2020;33(1):393-401.
10. Ozaslan M, Karagoz ID, Kilic IH, Guldur ME. Ehrlich ascites carcinoma. African Journal of Biotechnology. 2011; 10(13):2375-2378.
11. Sujana N, Ramanathan Santhanalakshmi, Vimala Venketela, Sundaram Meenakshi, Pemaiah B. Antitumour potential of passiflora incarnata against ehrlich ascites carcinoma. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(5):10-13.
12. Tousson E, Hafez E, Gazia MMA, Salem SB, Mutar TF. Hepatic ameliorative role of vitamin b17 against ehrlich ascites carcinoma–induced liver toxicity. Environmental Science and Pollution Research. 2020;1-11.
13. Sarkar R, Mandal N. In vitro cytotoxic effect of hydroalcoholic extracts of medicinal plants on ehrlich's ascites carcinoma (eac). International Journal of Phytomedicine. 2011;3(3):370.
14. Mutar TF, Gazia MA, Salem SB, Hammed EH, Tousson E. Ehrlich ascites carcinoma bearing mice as model of human hepatocellular carcinoma. Asian Journal of Research and Reports in Hepatology. 2019;1-9.
15. Vengadesan P, Ahmed John SG. Effects of bio chemical compounds in the in vitro anti-cancer activity of sargassum wightii from mandapam coast region of Tamil Nadu, India. 2017.
16. Patel MP, Patel RR, Patel JK. Chitosan mediated targeted drug delivery system: a review. Journal of Pharmacy & Pharmaceutical Sciences. 2010;13(4):536-557.
17. Aranaz I, Mengíbar M, Harris R, et Al. Functional characterization of chitin and chitosan. 2009;203-230.
18. Kumirska J, Mirko XW, Thöming J, Stepnowski P. Biomedical activity of chitin/chitosan based materials—influence of physicochemical properties apart from molecular weight and manuscript accepted accepted manuscript degree of n-acetylation. Polymers (Basel). 2011; 3:1875-1901.
19. Yang R, Shim WS, Cui FD, Cheng G, Han X, Jin QR, Kim DD, Chung SJ, Shim CK. Int J Pharm. 2009;371(1-2):142-7.
20. Mendonca C. Development and ex vivo characterization of enteric coated chitosan beads for crohn's disease management. 2018.
21. Turck D, Bresson JL, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, Mcardle HJ, Naska A. Efsa panel on dietetic products, nutrition and allergies (efsa nda panel). Symbiosal® and lowering of blood pressure and reduced risk of hypertension: EVALUATION of a health claim pursuant to article 14 of regulation (ec) no 1924/2006. Efsa Journal. 2018;16(7): 05364.
22. Moustafa AH, Ali EM, Moselhey SS, Tousson E, Elsaid KS. Effect of coriander on thioacetamide- induced hepatotoxicity in rats. Toxicology and Industrial Health. 2014;30(7):621- 9.
23. Alrasheed NM, Elmasry TA, Tousson E, Hassan HM, Alghadeer A. Hepatic protective effect of grape seed proanthocyanidin extract against gleevec induced apoptosis, liver injury and ki67 alteration sinrats. Brazilian Journal of Pharma Ceutical Sciences. 2018;54:(2).
24. El Moghazy M, Zedan NS, Elatrsh AM, Elgogary M, Tousson E. The possible effect of diets containing fish oil (omega3) on hematological, biochemical and histopathogical alterations of rabbit liver and kidney. Biomedicine & Preventive Nutrition. 2014;4(3):371-7.
25. Tousson E, Atteya Z, Elatrash E, Jeweely Oi. Abrogation by gink gobiloba leaf extract on hepatic and renal toxicity induced by methotrexate in rats. J Cancer Restreat. 2014;2(3):44-51.
26. Basuony M, Hafez E, Tousson E, Massoud A, Elsomkhraty S, Eldakamawy S. Beneficial role of panax ginseng root aqueous extract against cisplatininduced blood toxicity in rats. Am J Biol Chem. 2015;3(1):1-7.
27. El Atrash A, Tousson E, Gad A, Allam S. Hematological and biochemical changes caused by antidepressants amitriptyline induced cardiac toxicity in male rats. Asian Journal of Cardiology Research. 2019;1-6.
28. Amalraj A, Pius A, Gopi S, Gopi S. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives–a review. Journal of Traditional and Complementary Medicine. 2017; 7(2):205-233.

29. Ashokkumar D, Mazumder UK, Gupta M, Selvan VT. Effect on inhibition of proliferation and antioxidant enzyme level of lippia nodiflora in eac cell line treated mice. Journal of Complementary and Integrative Medicine. 2009;6(1).
30. El-Wahab, A., Samia, M, Fouda FM. Histological and histochemical study on the effect of ehrlich ascites carcinoma on the liver and kidney of mice and the possible protective role of tetrodotoxin. Egyptian Journal of Biology. 2009;11.
31. Adwas AA, Elkhoely AA, Kabel AM, Abdel-Rahman MN, Eissa AA. Anti-cancer and cardioprotective effects of indol-3-carbinol in doxorubicin-treated mice. Journal of Infection and Chemotherapy. 2016; 22(1):36-43.
32. Badr OM, Sakr S, Abd-Eltawab HA. Ameliorative effect of ginger extract against pathological alterations induced in mice bearing solid tumors. J. Biosci. Appl. Res. 2016;2(3):185-196.
33. Kim KT, Lee JY, Kim DD, Yoon IS, Cho HJ. Recent progress in the development of poly (lactic-co-glycolic acid)-based nanostructures for cancer imaging and therapy. Pharmaceutics. 2019; 11(6):280.
34. Adhikari HS, Yadav PN. Anticancer activity of chitosan, chitosan derivatives, and their mechanism of action. International Journal of Biomaterials. 2018.
35. Salem AH, Yassin AA, Hossam AT. Downregulation of transforming growth factor-β (tgf-β) and vascular endothelial growth factor (vegf) in ehrlich ascites carcinoma-bearing mice using stearic acid-grafted carboxymethyl chitosan (sa-cmc). Natural science. 2012.
36. Badr MO, Edrees NM, Abdallah AA, El-Deen NA, Neamat-Allah AN, Ismail HT. Anti-tumour effects of egyptian propolis on ehrlich ascites carcinoma. Vet Ital, 2011;47(3):341-350.

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