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The Potential Protective Effect of Spirulina Nanoparticles Against Ehrlich Solid Tumor Bearing Mice Induced Liver Toxicity, Tumor Markers, DNA Fragmentation, Oxidative Stress and Monooxygenase Variations

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Abstract

Ehrlich solid tumor (EST) is a spontaneous murine mammary adenocarcinoma, undifferentiated, making mainly beneficial for tumors studies. The current research aim to describe the antineoplastic activity of spirulina nanoparticles extract (SP NPs) against EST induced alteration in liver functions, oxidative stress, tumor markers, monooxygenase and DNA damage in female mice. 40 female mice were arbitrarily doled out into 4 gatherings (1st, Control; 2nd, SP NPs; 3rd, EST; 4th, EST + SP NPs). Results revealed significant elevation in the levels of AST, ALT, GGT, ALP, AFP, CEA, DNA damage; thiobarbituric acid reactive substances (TBARS), and glutathione peroxidase (GPx) in liver homogenate and depletions in the levels of albumin, total proteins, cytochromes (CYP450 & cyt b5), reduced glutathione (GSH), glutathione reductase (GR), glutathione S transferase (GST) and superoxide dismutase (SOD) activity in liver homogenate compared to control. EST Treatment with SP NPs (EST + SP NPs) improved and modulates these variations in liver functions, tumor markers, DNA damage, cytochromes, enzymatic and non-enzymatic, and oxidative stress as compared to mice bearing EST. These results suggest that SP NPs may have hepatic protective, antioxidant, and anti-cancer properties; in addition to inhibition of the EST development, and liver DNA damage induced by EST.

1. Introduction

Cancer is the most genuine motivation of death around the world, in 2016, 1.7 millon new malignancy (cancer) cases and 595690 disease passings are projected to occur in USA (Siegel et al., 2014). With 12.7 million cases in 2008, and it is normal that 27 million new cases will be analyzed in 2030. In addition, approximately 47% of disease cases and 55 % of its mortality occur in less created areas of the world (Jemal et al., 2011).

Breast disease is the second most incessant worldwide and the most well-known sort of malignancy among ladies, being liable for about 1.38 million new cases every year, and adding to around 458,000 passings, as per the World Health (DeSantis et al., 2014). Ehrlich tumor carcinoma is a transplantable, ineffectively separated dangerous tumor which showed up initially as an unconstrained bosom carcinoma in a mouse (El-Masry et al., 2019, 2020a; Abd Eldaim et al., 2021a). It fills in both solid and ascitic structures (Mutar et al., 2020; Alotaibi et al., 2021). At the point when the ascites liquids that is initially hyper diploid and it is 100% harm vaccinated intraperitoneal it created to EAC and whenever infused subcutaneously it created to EST (Abd Eldaim et al., 2019; Tousson et al., 2020).

Most methodologies treatments that utilized in disease therapy is chemotherapy that murder malignancy cells by instigating apoptosis and causes seriously influence the existence of patients and address an immediate reason for death (Tousson et al., 2014, 2016). Over 40% of remedial medications were delivered dependent on normal items and their subordinates in the previous many years (Alotaibi et al., 2020).

Spirulina is filamentous blue-green microalgae having a place with 2 distinct genera Spirulina and Arthrospira that comprises of around 15 species (Siva Kiran et al., 2015). In numerous nations of Africa, Spirulina used as food that full of protein and is accumulated from basic water, dried and eaten (Vedi et al., 2013). 70% of spirulina is protein in addition to carotenoid, omega 3 & 6, glycolipids, polysaccharides, sulfolipids, Gamma Linolenic Acid (GLA), provitamins; and many minerals as potassium, calcium, magnesium, iron, manganese, selenium and zinc (Khan et al., 2005). Pre-clinical and clinical assessments recommend that spirulina has certain helpful impacts as in decline in blood cholesterol, weight, radiation insurance, and nephrotoxicity (Deng and Chow, 2010; Siva Kiran et al., 2015).

Nanotechnology has an immense scope of utilization in conclusion, drug conveyance, food industry, ecological cleanup, paints, hardware, sportsbeauty care products, and sunscreens (Nel et al., 2006; El-Masry et al., 2020b). Nanoparticles (NPs) are materials with a size scope of roughly 1-100 nm (Altwaijry et al., 2020, 2021). The little size of these particles gives an expanded surface region and incites interesting and explicit physicochemical qualities like high conductivity, and compound reactivity contrasted with mass materials (Alotaibi et al., 2021). Along these lines; this exploration mean to portray the antineoplastic action of spirulina nanoparticles against Ehrlich tumor in the solid forms prompted adjustment in liver functions, oxidative stress, tumor markers, monooxygenase and DNA damage in female mice.

2. Materials And Methods

2.1. Substance and reagent

2.1.1. Spirulina nanoparticles synthesis

Spirulina nanoparticles (SP NPs) > 30 nm particle size were purchased from nanotech Company (Egypt Nanotech).

2.2. Ehrlich ascites carcinoma

Ehrlich ascites carcinoma (EAC) bearing mice were gotten from Egyptian National Cancer Institute in Cairo University.

2.3. Animals

A total of 40 Swiss albino mice (female; weighing 20–25 g) gotten from animal house colony Egypt vaccine company. The creatures were randomized and housed under surrounding room-temperature at 22–25 ^{0}C and relative stickiness conditions a 12-h light/12-h dim cycle, a business diet and water were given not indispensable to about fourteen days. The investigations were directed by rules gave by the Moral Committee of Faculty of Science at Tanta University and subject to underwriting by the Institutional Animal Care and Use Committee (IACUC – SCI – TU – 00173).

2.4. Experimental design and animal groups

Mice were similarly partitioned into four groups (Gp1 - Gp4):

Gp1: Control Gp in which mice didn't got any treatment.

Gp2: Spirulina nanoparticles Gp (Sp NPs) in which mice got Sp NPs (50 ng/kg bw/2day/) orally by stomach tube for about fourteen days.

Gp3: (EST); Ehrlich solid tumor Gp mice were infused hypodermically with about 3,000,000 cells of EAC/mouse weakened in buffer saline to start EST (Aldubayan et al., 2019).

Gp4: (EST + SP NPs); mice were infused hypodermically with about 3,000,000 cells of EAC per mouse to start tumor (EST) and left for about fourteen days till the improvement of tumor at that point treated with spirulina nanoparticles separate (SP NPs) for an additional fourteen days.

2.5. Sample collection

Before the finish of the analysis, mice were euthanized with intraperitoneal infusion with sodium pentobarbital and afterward went through complete necropsy. Blood tests from each mouse were gotten from the vena cava and accumulated in non-heparinised glass tubes prior to being left for 30 minutes to clump at 37C^{o} before their being liable to 5000 xg outward for ten minutes.

Sera were isolated and put away in aliquots at -80° C until required. Notwithstanding liver of each mouse was taken out, squashed and homogenized (10%, w/v) and the supernatant was gathered and put away at – 80°C for the assurance of DNA harm, oxidative pressure, microsomes and cytochromes.

2.6. Biochemical investigation

2.6.1. Liver functions

Total protein was estimated by the Biuret technique as depicted by Armstrong and Carr (1964), albumin was resolved by Doumas et al. (1971). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated by the strategy for Reitman and Frankel (1957) while Gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were estimated by the technique for Al-Rasheed et al. (2018) and Belfield and Goldberg (1971) respectively.

2.6.2. Assessment of tumor markers in serum

Alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA) was assessed through computerized utilizing the VIDAS® AFP and CEA quantitative protein connected fluorescent measure (ELFA) system (Biomerieux, Marcy-L'Etoile, France).

2.7. Determination of DNA breakages in liver homogenate

quantitative determination of the percentage of fragmentation of DNA in liver tissues was assessed using the technique of Wu et al. (2006) and Tousson et al. (2018) with some modifications.

2.8. Mixed-function monooxygenase systems in liver microsomes

The total microsomal hepatic substance of the cytochromes (cyt b5 and CYP450) was assessed by utilizing the strategy for Omura and Sato (1964). Amidopyrine N demethylase and aniline 4 hydroxylase activities were estimated by Nash (1953) and Kato and Gillette (1965), separately.

2.9. Liver homogenate biochemical assays

TBARS and GSH were estimated as depicted by Tappel and Zalkin (1959) and Pastore et al. (2003) respectively. Conversely; GST and GPx were measured by the strategy for Habig et al. (1974) and Paglia and Valentine (1967) respectively. In addition to SOD and GR were measured by the strategy depicted by Nishikimi et al. (1972) and Oyouni et al. (2018) respectively. Catalase was resolved by the strategy depicted by Saggu et al. (2014).

2.10. Statistical analysis

Mean and standard blunder esteems were resolved for every one of the boundaries and the outcomes were communicated as mean ± standard mistake. The information were investigated utilizing a single direction examination of difference (ANOVA) trailed by Duncan various correlation. $P < 0.05$ was statically huge as per Norušis (2006).

%change = Mean value of treated – Mean value of control / Mean value of control * 100

3. Results

3.1. Impacts of spirulina NPs on functions of liver

A significant elevation was assessed in the levels of ALT, AST, ALP, and GGT in EST when compared with control (Table 1). Conversely; a significant decline in the albumin and total proteins was estimated in EST when contrasted with control (Table 1). On the other hand, a significant decline was estimated in ALT, AST, ALP, and GGT in treated EST with spirulina nanoparticles (EST+SP NPS) (Table 1). Also, a significant elevation in albumin and total proteins in treated EST with spirulina nanoparticles (EST+SP NPS) (Table 1).

3.2. Impacts of spirulina NPs on tumor markers

A significant elevation were assessed in AFP and CEA levels in EST when compared with control (Table 2). On the contrary; a significant decrease in AFP and CEA levels in treated EST with spirulina nanoparticles (EST+SP NPS) were detected (Table 2).

3.3. Impacts in DNA breakages in liver tissue

A significant elevation in DNA breakages was assessed in liver tissues in EST group when contrasted with control (Table 3). In distinction, a significant decline in DNA breakages in liver tissues in treated EST with spirulina nanoparticles (EST+SP NPS) as compared to EST (Table 3).

3.4. Effects of spirulina NPs on drug metabolism

The cytochromes (CYP450 & cyt b5), amidopyrine N demethylase and aniline 4 hydroxylase in mice liver showed normal activity in control and SP NPs groups (Table 5). Ehrlich solid tumor induced depletion in cytochromes (CYP450 & cyt b5) and elevation in Aniline4-hydroxylase as compared to control. Conversely; treatment of EST with spirulina nanoparticles (EST+SP NPS) induced elevation in cytochromes (CYP450 & cyt b5) and depletion in Aniline4-hydroxylase as compared to EST (Table 4).

3.5. Changes in oxidative stress in liver tissue homogenate

A significant elevation was distinguished in TBARS, and GPx, in liver homogenates in EST group when contrasted with control (Table 4). Conversely; a significant decline in TBARS, and GPx in liver in treated EST with spirulina nanoparticles (EST+SP NPS) (Table 5). A significant decline in GSH, GR, GST and SOD activities in liver homogenate in EST group as compared to control group were detected (Tables 4). In contrast; a significant elevation in GR, GST and SOD in liver homogenate in treated EST with spirulina nanoparticles (EST+SP NPS) (Table 5).

4. Discussion

Malignant growth is a primary general medical condition worldwide and is the subsequent driving reason for death. Ebb and flow research plan to portray the antineoplastic action of spirulina nanoparticles separate against Ehrlich solid tumor actuated modification in liver functions, oxidative stress, tumor markers, monooxygenase and DNA damage in female mice. Our outcomes uncovered that; EST induced elevations in AST, ALT, ALP, and GGT, and exhaustions in total protein and albumin suggesting liver dysfunction. Notwithstanding; the treatment of EST with SP NPs (EST + SP NPs) improved the liver capacities. The height of liver functions (AST, ALT, ALP, and GGT) is a list of weakening hepatic capacities because of malignancy expansion as seen in EST. These results are in concordance with aftereffects of Aldubayan et al. (2019) and El-Masry et al. (2020a). The discoveries here are reliable with those of El-Sheekh et al. (2014) who examined the defensive impacts of Spirulina on the liver functions and hyperlipidemia of rodents and human. Likewise; our outcomes concur with Torres-Durán et al. (1999) who concentrates on the preventive impact of Spirulina maxima on greasy liver improvement prompted via carbon tetrachloride, in the rodent.

In the current examination; EST actuated heights in tumor marker AFP and CEA mirror the fiery movement in mice and the post medicines with SP NPs consumption the rises in AFP and CEA levels. This affirmed

the discoveries of Choi and Kakar (2017), Aldubayan et al. (2019) and El-Masry et al. (2020a) who revealed that the expansion of AFP and CEA levels in serum can be an impression of hepatic incendiary movement and might be related with height AST, ALT and ALP catalysts in serum. The discoveries here are steady with those of Tousson et al. (2020) who find that; EAC induced significant increase in plasma AFP and vitamin B17 able to improve these changes.

Current outcomes uncovered that; EST prompted DNA damage in liver tissues and SP NPs ready to improve this harm. The discoveries here are reliable with those of Tousson et al. (2020) and Mutar et al. (2020) who find that; EAC initiated damage in the tissues of liver and kidney individually and the medicines with vitamin B17 can improve this harm. Also; current results agree with Abd Eldaim et al. (2019, 2021b) who reported hat; EST actuated variations in DNA in mice kidney and liver and the medicines with proanthocyanidin able to improve these damages.

The blended capacity oxidase or monooxygenase is likewise called liver medication processing proteins which utilize most medications, xenobiotics and cancer-causing agents into lower or more dynamic middle of the road (Sheweita, 2000). In the current study; depletion in cytochromes (CYP450 & cyt b5) and elevation in Aniline 4 hydroxylase were detected in EST. These results suggest selective damage in the host tissue mitochondrial metabolism. Also; the treatments with SP NPs modulates the monooxygenase enzymes and improved the toxicity in the liver. However, the presence of SP NPs (Co-treated) group leads to elevate and modify the cytochromes. The discoveries here are steady with those of Tousson et al. (2020) who find that; EAC induced significant decrease in cytochromes in liver tissues.

Oxidative stress is firmly identified with all parts of disease, from carcinogenesis to the tumor bearing state, from treatment to anticipation (El-Demerdash et al. 2018). A few examinations have demonstrated that tumor development can cause cancer prevention agent aggravations and speeds up lipid peroxidationin indispensable organs of the tumor has (Alotaibi et al., 2021; Ibrahim et al., 2011). Our outcomes uncovered a huge rise in TBARS, GPx, and a huge decrease in GSH, GR, GST, SOD exercises in liver homogenates in EST when contrasted with control. Our outcomes concur with Ali et al. (2015) and El-Masry et al. (2019) who find that EST actuated height in MDA and decrease in GSH, catalase and SOD levels in blood and liver.

Present examination exhibited that; the medicines of EST with SP NPs was powerful in controlling cell reinforcement chemical exercises by decrease the degrees of TBARS, GPx and a critical height in GSH, GR, GST, SOD exercises. Spirulina contains a significant compound SOD that is 1700 units/g of dry mass that acts in a roundabout way by hindering the pace of oxygen revolutionary producing responses (Belay, 2002). Besides, Karadeniz et al. (2009) tracked down that; Pre-treatment with Spirulina platenesis may assume a part in lessening the poisonous impact of cadmium and its cancer prevention agent properties appeared to intervene a particularly defensive impact, shown by the decrease of MDA and NO just as the rise in levels of GSH and SOD. This is likewise in accordance with Hassanen et al. (2015) who consider the biochemical impacts of spirulina platensis against oxidative pressure brought about by doxorubicin.

5. Conclusions And Recommendations

Spirulina nano-particles have ability to improve liver functions and structure, inhibit the proliferations, AFP, CEA, and modulate the oxidative stress, electrolytes, DNA damage in breast cancer female mice. Whenever affirmed in people's creatures, these outcomes could demonstrate that spirulina nano-particles, as cell reinforcement, could be utilized as an assistant preventive treatment with chemotherapy drugs.

Declarations

Authors' contributions:

Gihan H Abd Elsamie, Sabah G El-Banna, Ehab Tousson proposed the idea and the experimental design; Omar NK Alheeti conducted the experiment and wrote the draft of manuscript with the help of Gihan H Abd Elsamie, Sabah G El-Banna, Ehab Tousson supervised the implementation of the experiment and analyzed the results. All authors discussed the results and contributed to finalize the manuscript.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Data availability:

All data used in this study are included in this published article.

Compliance with ethical standards

Conflict of interest:

All authors declared that they have no conflicts of interest regarding the publication of this manuscript.

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Ethical approval:

Rearing and treatment of mice and all experimental procedures were conducted according to the guide for the animal use that approved by Institutional Animal Care and Use Committee (IACUC-SCI-TU-00173), Faculty of Science, Tanta University.

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Tables

Table 1

Changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AlP), gamma glutamyl transferase (GGT), total protein (TP) and Albumin in sera of female mice.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	T. Protein (g/dL)	Albumin (g/dL)
Control	126.60 ^{b}±3.91	32.0 ^{b} ± 1.48	146.2 ^{c} ± 3.40	6.03^{b} ± 0.29	6.07 ± 0.03^{a}	4.54 ± 0.07^{a}
% change	–	–	–	–	–	–
SP NPS	125.40^{b} ± 3.36	29.80 ^{b} ± 0.80	139.4 ^{c} ± 1.57	5.89 ^{b} ± 0.29	6.04 ± 0.07^{a}	4.59 ± 0.05^{a}
% change	-0.95	-6.88	-4.65	–2.32	-0.49	1.10
EST	165.6 ^{a} ± 2.11	54.8 ^{a} ± 2.76	207.20^{a} ± 4.43	7.99 ^{a} ± 0.27	5.26 ± 0.06^{c}	3.61 ± 0.11^{b}
% change	30.81	71.25	41.72	32.50	-13.34	-20.48
EST+SP NPS	137.80^{b} ± 4.02	33.60 ^{b} ± 1.57	176.80^{b} ± 6.37	7.16 ^{a} ± 0.21	5.81 ± 0.06^{b}	4.06 ± 0.08^{c}
% change	8.85	5.0	20.93	18.74	-4.28	-10.57

The results expressed as (Mean ± SE). The number of the mice in each group is ten Means in the column with Common letters are not significant (i.e. Means with different letters are significant). (SP NPs, Spirulina nanoparticle; EST, Ehrlich solid tumor).The results expressed as (Mean ± SE). The number of the mice in each group is ten Means in the column with Common letters are not significant (i.e. Means with different letters are significant). (SP NPs, Spirulina nanoparticle; EST, Ehrlich solid tumor).

Table 2

Changes in Alpha- fetoprotein (AFP) and carcinoembryonic antigen (CEA) in blood serum of female mice

Groups	AFP (ng/mL)	CEA (ng/mL)
Control	0.16 ± 0.03^{c}	0.29 ± 0.02^{c}
% change	–	–
SP NPS	0.14 ± 0.01^{c}	0.24 ± 0.02^{c}
% change	−12.50	−17.24
EST	0.99 ± 0.04^{a}	0.88 ± 0.04^{a}
% change	518.75	203.45
EST+SP NPS	0.33 ± 0.03^{b}	0.57 ± 0.03^{b}
% change	106.25	96.55
The results expressed as (Mean ± SE). The number of the mice in each group is ten. Means in the column with Common letters are not significant (i.e. Means with Different letters are significant). (SP NPs, spirulina nanoparticle; EST, Ehrlich solid tumor)The results expressed as (Mean ± SE). The number of the mice in each group is ten. Means in the column with Common letters are not significant (i.e. Means with Different letters are significant). (SP NPs, spirulina nanoparticle; EST, Ehrlich solid tumor)		

Table 3

Changes in DNA breakages in liver of female mice

Groups	DNA breakages (%)
Control	5.38 ± 0.19^{bc}
% change	–
SP NPS	4.91 ± 0.19^{c}
% change	−8.74
EST	7.91 ± 0.35^{a}
% change	47.03
EST+SP NPS	6.49 ± 0.44^{b}
% change	20.63
The results expressed as (Mean ± SE). The number of the mice in each group is ten. Means in the column with Common letters are not significant (i.e. Means with Different letters are significant). (SP NPs, spirulina nanoparticle; EST, Ehrlich solid tumor)The results expressed as (Mean ± SE). The number of the mice in each group is ten. Means in the column with Common letters are not significant (i.e. Means with Different letters are significant). (SP NPs, spirulina nanoparticle; EST, Ehrlich solid tumor)	

Table 4

Changes in Cytochrome b_5 (nmol cytochrome/mg protein), Cytochrome P_{450} (nmol cytochrome/mg protein), AmidopyrineN-demethylase (µmol/min x kg liver sample) and Aniline4-hydroxylase (µmol/min/mg protein) in liver of female mice

Groups	Cytochrome b_5	Cytochrome P_{450}	AmidopyrineN-demethylase	Aniline4-hydroxylase
Control	5.11 ± 0.32[a]	6.84 ± 0.13[a]	0.098 ± 0.024[a]	0.113 ± 0.028[b]
% change	-	-	-	-
SP NPS	4.88 ± 0.19[a]	6.88 ± 0.15[a]	0.122 ± 0.003[a]	0.139 ± 0.002[ab]
% change	-4.50	0.58	24.49	23.01
EST	3.66 ± 0.10[b]	5.64 ± 0.17[b]	0.116 ± 0.003[a]	0.181 ± 0.001[a]
% change	-28.38	-17.54	18.37	60.18
EST+SP NPS	3.13 ± 0.08[b]	4.55 ± 0.18[c]	0.123 ± 0.001[a]	0.132 ± 0.001[ab]
% change	-38.75	-33.48	25.51	16.81

The results expressed as (Mean ± SE). The number of the mice in each group is ten

Means in the column with Common letters are not significant (i.e. Means with Different letters are significant). (SP NPs, spirulina nanoparticle; EST, Ehrlich solid tumor)The results expressed as (Mean ± SE). The number of the mice in each group is ten

Means in the column with Common letters are not significant (i.e. Means with Different letters are significant). (SP NPs, spirulina nanoparticle; EST, Ehrlich solid tumor)

Table 5

Changes in thiobarbituric acid reactive substances (TBARS), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), reduced glutathione (GSH) and superoxide dismutase (SOD) in liver homogenate of female mice

Groups	TBARS (µmol/g tissue)	GR (IU/g tissue)	GPx (IU/g tissue)	GST (IU/g tissue)	GSH (mg/g tissue)	SOD (IU/g tissue)
Control	3.35 ± 0.10^{c}	40.76 ± 0.60^{a}	132.21 ± 2.29^{c}	5.42 ± 0.32^{a}	149.43 ± 7.51^{b}	1366.41 ± 22.42^{a}
% change	–	–	–	–	–	–
SP NPS	3.25 ± 0.28^{c}	38.28 ± 1.54^{a}	162.71 ± 5.93^{b}	6.08 ± 0.41^{a}	159.26±12.64^{b}	1369.92 ± 28.25^{a}
% change	-2.99	-6.08	23.07	12.18	6.58	0.26
EST	4.76 ± 0.11^{a}	18.44 ± 0.65^{c}	207.06 ± 6.91^{a}	3.41 ± 0.32^{b}	101.15 ± 3.15^{a}	1301.95 ± 17.22^{a}
% change	42.09	-54.76	56.61	-37.08	-15.61	-4.72
EST+SP NPS	4.12 ± 0.26^{ab}	25.27 ± 1.22^{b}	175.62 ± 9.77^{b}	5.98 ± 0.29^{a}	132.57±12.67^{b}	1375.78 ± 33.47^{a}
% change	22.99	-38.0	32.83	10.33	-11.28	0.69

The results expressed as (Mean ± SE). The number of the mice in each group is ten

Means in the column with Common letters are not significant (i.e. Means with Different letters are significant). (SP NPs, spirulina nanoparticle; EST, Ehrlich solid tumor)The results expressed as (Mean ± SE). The number of the mice in each group is ten

Means in the column with Common letters are not significant (i.e. Means with Different letters are significant). (SP NPs, spirulina nanoparticle; EST, Ehrlich solid tumor)

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