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ANTITUMOR ACTIVITY, HEMATOXICITY AND HEPATOTOXICITY OF SORAFENIB FORMULATED IN A NANOEMULSION BASED ON THE CARROT SEED OIL

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ABSTRACT

Combining the chemotherapeutic agent, sorafenib (SRF), with the essential oil, carrot seed oil, can help in reducing the adverse side effects of the SRF. However, the different solubilities of both components impede the mixing, which can be overcome by formulating the carrot oil in a nanoemulsion (NANO). The aim of the present study was to evaluate the anticancer activity, hematoxicity and hepatoxicity of SRF when mixed with the nanoemulsion formulated of the carrot oil (NANO-SRF). As measured by the zetasizer, it has been found that the dispersed nanodroplets of the formulated NANO and NANO-SRF have z-average diameters of 10.27 ± 2.39 nm and 68.92 ± 10.6nm, respectively. Forty female Swiss Albino mice bearing Ehrlich ascites carcinoma (EAC) were split into four groups (n =10). Group I served as the untreated EAC mice while groups II-IV were administered via oral gavage with the 30 mg/kg mouse of SRF solubilized in 0.2 mL of Chremophore/ethanol solution, 30 mg/kg mouse of SRF solubilized in 0.2 mL of NANO and 0.2mL of drug –free NANO, respectively. The results of the antitumor assessment revealed that the GPX and LDH activities in the ascetic fluid of III-NANO-SRF group were enhanced when compared to II-SRF group. The white blood cell counts reduced while the number of the platelets increased for III-NANO-SRF group when compared to II-SRF group. The amounts of alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T.BIL) and direct bilirubin (D. BIL) of the mice treated with the NANO-SRF were ameliorated when compared to the mice treated with the SRF formula. Mixing the SRF with the NANO has improved the efficacy of SRF while reducing its hematoxicity and hepatotoxicity.

KEYWORDS : *Anticancer drugs; Tyrosine kinase antagonist; Zetasizer, Zeta potential, Z-average diameter; Ehrlich ascites carcinoma*

INTRODUCTION

Cancer is generally defined as the abnormal proliferation of cells that could metastasize to the nearby tissues.[1]In fact, cancer treatment via chemotherapeutic agents has many obstacles, especially in weak and older patients due to the non-selectivity of the drugs that may target the healthy organs.[2-3]Sorfenib (SRF), a tyrosine kinase antagonist, is mainly used clinically in treating hepatocellular carcinoma and renal cell carcinoma. However, patients treated with SRF were found to experience skin rashes in their hands and feet, exhaustion, diarrhea and hypertension.[4] Therefore, many research studies have suggested that combining SRF with other agents would eliminate its side effect .[5]Additionally, SRF may be combined with other essential oils that have beneficiary properties such as antitumor, anti-inflammatory and/or antimicrobial.[6] Carrot seed essential oil, extracted from *D. carota* seeds of plant *Daucus carota* by steam distillation, has anticancer activities due its constituents.[7]It may contain α-pinene, β- bisabolene, β-pinene, γ-terpinene, camphene, carotol, geranyl acetate, limonene, myrcene and sabinene as main chemical constituents.[8] Due to the hydrophobicity of the carrot oil as many essential oils, it is more suitable to solubilize it in nanoemulsions, colloidal systems that mix the liquids with large differences in their surface tensions by the mean of the surfactants and cosurfactants.[9] It has been reviewed recently that the essential oils which have antimicrobial activity were mixed with food through formulation in nanoemulsions.[10]The objective of the present study was to *in vivo* assess the anticancer activity of the

SRF mixed with a nanoemulsion formulated with the carrot oil.

MATERIALS AND METHODS

Materials and subjects

Carrot seed oil was purchased from Sokar Nabat for Natural Oils (Jeddah, KSA). Span 20 and Tween 80 were obtained from Al-Rowad modern establishment for the supply of medical equipment (Jeddah, KSA). SRF was procured from Dr.Nermin Pharmacy (Cairo,Egypt). All of the assay kits were purchased from Bioassays for diagnostic and research reagents (Hayward, USA), the Crescent diagnostics Company(Saudi Arabia) and Human Biochemical and Diagnostic (Wiesbaden, Germany). Forty female Swiss Albino mice (average weight 25-30 g) were acclimatized in accordance with King Abdulaziz University's policy and the International Ethical Guidelines for the care and use of laboratory animals.[11]The ethical approval was obtained from the research ethics committee in the Faculty of Medicine at King Abdulaziz University (PS-38-1984).

Preparation and zetasizer measurements of the NANO formulations

The drug-free NE formulation (NANO) was prepared by mixing 6.7% (v/v) of surfactant mixtures of Span 20 and Tween 80 at a ratio of 1:2, respectively, 89.7% (v/v) of carrot oil and 3.6% (v/v) of distilled water. Then, the mixture was vortexed with continuous heating in a water bath for one week at 70°C. The SRF-loaded-NE (SRF-NANO) was prepared by directly dissolving 30 mg of SRF/ kg of mouse body weight in 0.2 mL of NANO. Another formula for the SRF solution (SRF) was prepared by solubilizing 30 mg/kg of mouse in 0.2 mL of a solution containing a fixed ratio of 1:1 of Cremophor and 95% Ethyl Alcohol.The size and charge of the NE droplets (NANO-SRF and NANO) were measured by using Zetasizer Nano ZS (version no MAN0487-2-0, Malvern Instruments, UK).

In vivo antitumor activity of drug formulations

The Ehrlich ascites carcinoma (EAC) cells were inoculated in the peritoneum cavity of the mice as mentioned by Alkhatib *et al*. [12]After splitting the 40 mice into four groups (n =10), each mouse was weighed followed by injecting it with 2.5×10^6 EAC cells. After 48 hours, groups II-IV were administered with SRF, SRF-NANO and NANO, respectively, at a dose of 30 mg/kg of mouse every other day for a total of 6 doses via oral gavage administration as shown in Table 1.[13]The amount of food consumed by the mice was monitored daily for two weeks by subtracting the amount of food remaining from the initial amount of served food for each cage (100g). On the 15th day, mice were weighed and sacrificed after keeping them fasting for 12h. After that, each mouse's ascetic fluid and blood were collected for the biochemical analysis. Additionally, the liver organ was resected and weighed to measure the relative liver weight (RLW) which was calculated by dividing the liver weight by the mouse body weight.

Table 1
The tested animal groups, containing 10 mice, with their administered treatment.

Group No.	Group name	Administered treatment	Doses
I	EAC	-	
II	SRF	SRF	30mg/kg of mouse dissolved in 0.2 mLCremophor
III	NANO-SRF	SRF-NANO	30mg/kg of mouse dissolved in 0.2 mL NANO
IV	NANO	NANO	0.2mL NANO

LDH and GPX activities in the ascetic fluid

The supernatant of the collected fluid was prepared as described elsewhere.[14]The assessment of LDH activity was performed according to the protocol of LDH LR (SCE MOD, Cat.No. CZ 908 L) kit. The GPX assay was implemented as mentioned elsewhere.[15]

Hematological parameters

The collected blood was used for the estimation of white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb) and platelets by using complete blood count (CBC) analyzers, purchased from Beckman Coulter, California, U.S.

Biochemical parameters

Following clotting of the collected blood samples, serum was separated by centrifugation at 3000 RPM for 15 min. Serum was utilized for the detection of liver function in terms of measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T.BIL), direct BIL (D.BIL), total protein (TP) and albumin (ALB) by using the optimized UV-test according to the International Federation of Clinical Chemistry (IFCC) (Crescent diagnostics Company, Saudi Arabia).

STATISTICAL ANALYSIS

All values obtained from the experiments (n=10) were expressed as mean ± standard deviation. Statistical analysis was performed with one-way analysis of variance (ANOVA) test and independent sample t-test using the MegaStat Excel (version 10.3, Butler University). The significant difference was considered when $p < 0.05$.

RESULTS

Physical characterization of the NANO formulations

Zetasizer measurements are illustrated in Table 2. The z-average diameter of the NANO formula has significantly increased when loaded with SRF (SRF-NANO) ($P = 0.0007$). The variations between the droplet diameters of both formulas were minimal as their percentages of coefficient of variations were less than 25%. Although the values of the zeta potentials of both formulas were comparable ($P= 0.0734$), their charges have differed.

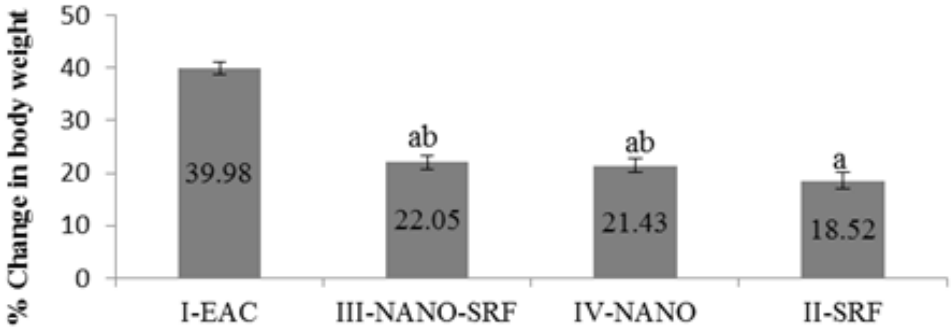
Table 2
The zetasizer measurements of the NANO and SRF-NANO formulations. Data were expressed as mean ± standard deviation.

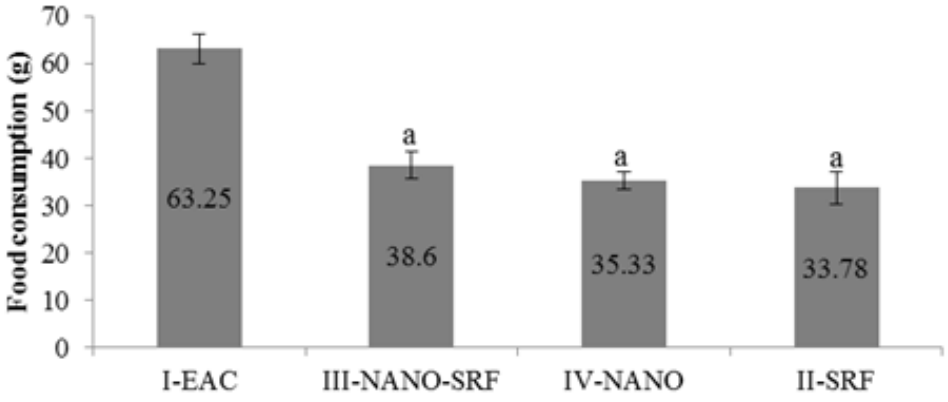
Formulation	Z-Average diameter (nm)	Zeta Potential (mV)	% Coefficient of variation
NANO	10.27 ± 2.39	0.124 ± 0.018	23.20
NANO - SRF	68.92 ± 10.6	-0.815 ± 0.674	15.30

Anticancer activity of the administered drugs

Body weight change and food consumption

As displayed in Figure 1, there is a direct proportion between the % change in body weight and the average amount of food consumed by the mice per day during 15 days. In particular, the maximum increase in body weight and the amount of food consumed were observed in I-EAC group, whereas the least were recorded in II-SRF group. In III-NANO-SRF and IV-NANO groups, the amount of food consumed and the % increase in body weight were slightly greater than II-SRF group but they were remarkably less than I-EAC group.





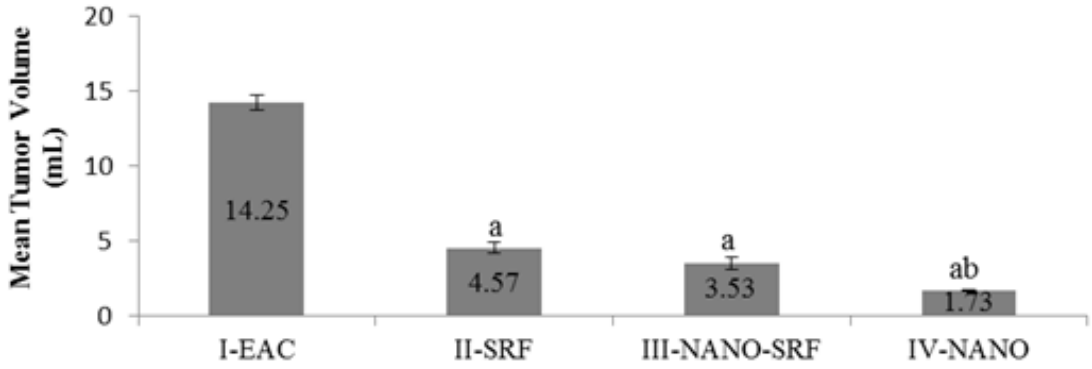
Error bars display the standard error of the mean. [a] There is a significant difference between I-EAC group and the individual group; [b] there is a significant difference between II-SRF group and the individual group

Figure 1

The effect of the drug formulations on the % change in the body weight and the mean amount of food consumption of mice per day during 15 days.

LDH and GPX activities in the ascetic fluid

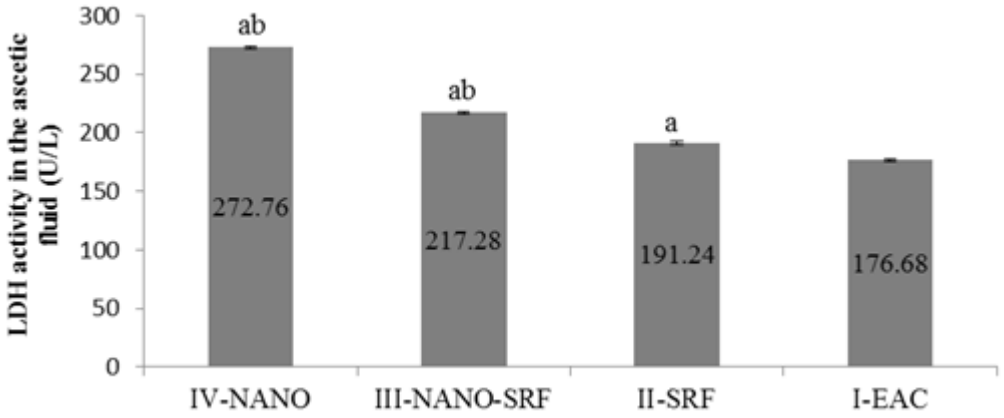
In terms of the tumor growth, as shown in Figure 2, the largest mean tumor volume was collected from the peritoneal cavity of I-EAC group, whereas the least amount was collected from the IV-NANO group. It should be noted that the amount of the ascetic fluid collected from the peritoneal cavities of III-NANO-SRF and IV-SRF groups were comparable. Regarding the LDH and GPX activities in the ascetic fluid, as exhibited in Figures 3 and 4, they were greater in III-NANO-SRF and IV-NANO groups than in I-EAC and II-SRF groups. Interestingly, the GPX and LDH activities in the ascetic fluid were the highest in IV-NANO group among all of the tested groups.



Error bars display the standard deviation. [a] There is a significant difference between I-EAC group and the individual group; [b] there is a significant difference between II-SRF group and the individual group

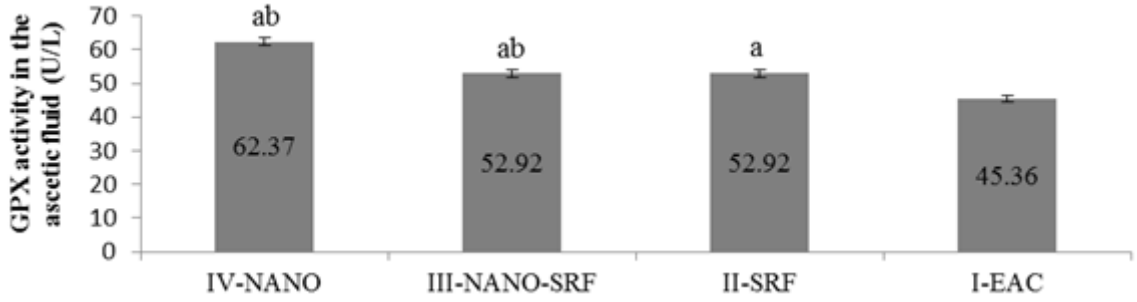
Figure 2

The effect of the drug formulations on the mean tumor volume (mL).



Error bars display the standard deviation. [a] There is a significant difference between I-EAC group and the individual group; [b] there is a significant difference between II-SRF group and the individual group

Figure 3
The effect of the drug formulations on the LDH activity in the ascetic fluid.



Error bars display the standard deviation. [a] There is a significant difference between I-EAC group and the individual group; [b] there is a significant difference between II-SRF group and the individual group

Figure 4
The effect of the drug formulations on the GPX activity in the ascetic fluid.

Effect of drug formulations on the hematological parameters of the mice

The hematological parameters of the tested mice were determined as illustrated in Table 3. The WBCs counts have considerably decreased in III-NANO-SRF and IV-NANO groups when compared to II-SRF group. In fact, the WBCs count of IV-NANO was significantly less than that of I-EAC.In terms of the RBCs counts and levels of Hb, there were no significant differences between all of the tested groups. However, the amount of platelets of III-NANO-SRF groupwas the highest among all of the tested groups. Interestingly, they have significantly decreased in the IV-NANO group when compared to the II-SRF andIII-NANO-SRF groups.

Table 3
The effect of drug formulations on the hematological parameters of the experimental groups. Data were expressed as mean ± standard deviation

Groups	Total WBCs cells/µL	Total RBCs cells/µL	Hb (g/dL)	Platelets ($\times 10^3$ cells/µL)
I-EAC	12.07±1.37	6.85±1.50	12.60±1.87	560±109
II-SRF	13.3±1.98	7.30±1.25	12.00±1.64	900±208[a]
III-NANO-SRF	9.43±1.18[b]	7.46±1.51	13.17±1.87	1401±93[ab]
IV-NANO	7.43±1.55[ab]	6.73±1.40	12.40±1.73	667±95[b]

[a] There is a significant difference between I-EAC group and the individual group; [b] there is a significant difference between II-SRF group and the individual group.

Effect of drug formulations on the liver function of the mice

Table 4 illustrates all of the parameters measured in the tested mice to detect their liver function. The RLW was enlarged in II-SRF group when compared to all of the tested groups. The levels of ALT have considerably enhanced in II-SRF and III-NANO-SRF groups when compared to I-EAC and IV-NANO groups. In fact, the level of ALT of III-NANO-SRF was less that of II-SRF group. On the other hand, there were no significant variations in the levels of AST among all of the tested groups. In terms of ALP levels, they have markedly increased in all of the treated groups when compared to I-EAC group. In particular, the level of ALP of II-SRF group was higher than the levels of III-NANO-SRF and IV-NANO groups. The amounts of T.BIL and D.BIL were the least in the IV-NANO group among all of the tested groups, but they were the highest in II-SRF group. However, the amounts of TP and ALB did not considerably differ in all of the tested groups.

Table 4

The effect of the drug formulations on the liver function of the mice. Data were expressed as mean ± standard deviation.

Parameter	I-EAC	II-SRF	III-NANO-SRF	IV-NANO
RLW	0.042±0.013	0.060±0.011[a]	0.048±0.007	0.047±0.005
ALT (U/L)	12.13±1.09	16.31±1.04[a]	14.98±1.27[ab]	12.55±1.52[b]
AST (U/L)	13.62±1.43	15.30±1.55	14.42±1.75	13.64±1.12
ALP (U/L)	61.26±2.89	104.32±1.97[ab]	94.65±1.21[ab]	80.02±2.4[ab]
T.BIL (mg/dL)	0.63±0.13	0.83±0.19	0.73±0.04	0.43±0.07[b]
D.BIL (mg/dL)	0.22±0.01	0.27±0.02	0.25±0.07	0.16±0.01[b]
TP (g/dL)	8.04±1.48	7.98±1.21	7.85±1.43	8.67±1.75
ALB (g/dL)	4.13±0.22	4.01±0.21	3.97±0.01	4.41±0.57

[a] There is a significant difference between I-EAC group and the individual group;
[b] there is a significant difference between II-SRF group and the individual group.

DISCUSSION

The current study found that the NANO formula has antitumor activity since the mice bearing Ehrlich carcinoma in their ascites, administered with the NANO (IV-NANO), has the smallest mean tumor volume with the largest LDH and GPX activities, enzymes that can be used as an indicator of the cell's damage.[16,17]This activity can be attributed to the presence of carrot oil, which has been found previously that one of its major component, carotol, has antiproliferative effect in the myeloid leukemia cancer cells [18], colon cancer cells and breast cancer cells.[19,20]In addition, incorporation of the carrot oil into positively charged nanodroplets improve its cellular permeation and uptake.[21,22]The nanoemulsion encapsulation provide protection for the carrot oil from degradation by the digestive enzymes and thereby enhance their intestinal absorption.[23] Combining SRF with the NANO has improved the SRF anticancer activity as the activity of the GPX and LDH in the ascetic fluid of III-NANO-SRF group has enhanced, although the mean tumor volume did not significantly differ from II-SRF group. Mixing SRF with the NANO formula has enlarged the nanodroplet size and change the charge of the droplet to negative, which could result in reducing the antitumor effect of the NANO while increasing the effect of SRF. In 2012, Fröhlich[24] has reviewed that the surface charge of the various nanoparticles may affect their cytotoxicity. In terms of the effect of the drug formulations on the hematological parameters of the mice, it has been found that the WBCs were mostly reduced in IV-NANO group when compared to the I-EAC group. In addition, combining SRF with the NANO has reduced the increased WBCs count caused by SRF. It should be noted here that NANO formula reduced the inflammation caused by the cancer due to the presence of the anti-inflammatory, α-pinene, in the carrot oil.[25] Furthermore, the combination of SRF with the NANO has increased the platelets without affecting the Hb and RBCs count which could be beneficial since SRF was found to cause thrombocytopenia in some clinical trials.[26,27] The toxic effect of the drug formulas on the livers of the mice was very obvious in the mice treated with SRF as the RLW was enlarged and the levels of ALT and ALP were elevated. However, mixing SRF with the NANO has reduced the liver inflammation caused generally by SRF.[28] Interestingly, the amounts of ALT, ALP, T.BIL and D. BIL of the mice treated with the NANO were ameliorated when compared to the mice treated

with the SRF formulas. It has been found that the carrot oil, one of the major components of the NANO formula, has hepatoprotective property in the mice administered with CCl_4, which is used to cause hapatotoxicity.[29]Additionally, one of the components of carrot oil, Kaempferol, was found to eliminate the toxic effect of the paracetamol on the liver.[30]

CONCLUSIONS

Combining the SRF with the carrot seed oil formulated in a nanoemulsion had potentiated the efficacy of SRF while reducing its adverse effect on the liver and blood. The drug-free NANO formula has potential antineoplastic activity that was greater than SRF solution and NANO-SRF formula. Additionally, it did not have a hematoxicand a hepatotoxic effect like the SRF solution. Further studies have to be performed to investigate the effect of the NANO formula on the other organs.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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