Promising antitumor activity of fermented wheat germ extract in combination with selenium nanoparticles

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ABSTRACT

Fermented wheat germ extract (FWGE) is a multi-substance composition and currently used as nutrition supplement for cancer patients. Nanotechnology holds promise for medication and nutrition because materials at the nanometer dimension exhibit novel properties different from those of both isolated atom and bulk material. Selenium nano particle (Nano-Se) is a novel Se species with novel biological activities and low toxicity. The aim of our study is to evaluate antitumor activity of fermented wheat germ extract and fermented wheat germ extract in combination with selenium nanoparticles (FWGE-nano-Se mixture). The two prepared materials were applied on an experimental carcinogenesis model in order to evaluate their *in vitro* and *in vivo* antitumor potential; against animal carcinogenesis "Ehrlich carcinoma". Cytotoicity assay of different concentrations of FWGE and FWGE-nano-Se mixture on EAC cells was evaluated by trypan blue exclusion method. *In vivo* studies were done by induction of solid tumors produced by intramuscular inoculation of EAC in the right thigh of the lower limb of each mouse and treating Erlich tumor bearing mice orally with FWGE and FWGE-nano-Se mixture for 6 weeks. Tumor volume was determined all over the experimental period. Blood, liver and tumor tissue samples were collected after 2 and 6 weeks from the beginning of treatment. The production of $NO_{(X)}$, MDA,CAT,SOD,GSH , GPx, ALT,AST,GGT (as liver function test), urea, and creatinine (as kidney function test) were evaluated by colorimetric assays, also, histopathological examination of liver and tumor tissue and characterization of cell death within tumor tissue was evaluated. *In vitro* results showed treatment of EAC cells with different concentrations of FWGE (0.21-85 mg/ml) showed cytotoxicity with IC_{50} at concentration of 0.8mg /ml , and in case of FWGE-nano-Se, showed cytotoxicity with IC_{50} at concentration of 0.8 mg /ml FWGE +0.75 µg /ml nano-Se. Also, *in vivo* studies results of FWGE-nano-Se mixture treated group showed significant reduction in the tumor volume compared to positive control group and FWGE treated group. Moreover, results of antioxidant parameters showed significant increase in SOD, GSH, GPx and CAT and significant decrease $NO_{(X)}$and MDA and improvement in liver and kidney function tests. Apoptosis and histopathological examination revealed that FWGE-nano-Se mixture has antimetastatic effect and induced apoptosis in Ehrlich carcinoma cells. We concluded that the anti-tumor mechanisms of FWGE-nano-Se may be mediated by preventing oxidative damage, improved liver and kidney function, decrease metastases of cancer cells and increae apoptosis. So FWGE-nano-Se might be a potential alternative agent for cancer therapy.

Clinical trials will be needed to spur the development of FWGE-nano-Se as cancer therapeutic agents.

Key words: Fermented wheat germ extract,selenium nanoparticles, cytotoxicity, Ehrlich Ascites Carcinoma Cells, tumor.

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INTRODUCTION

Nanotechology holds promise for medication and nutrition because materials at the nanometer dimension exhibit novel properties different to those of both isolated atoms and bulk material **[1].** Nanoparticles are designed to carry anti-cancer drugs and bring that medication all the way to the diseased cells in a person's body without harming the healthy cells **[2].** Selenium, as one of the essential elements for the health of mammalian animals, has key functions in the balancing of the redox system, the proper functions of the immune system, and anticarcinogenetic effects **[3]**. Nano-Se can serve as an antioxidant with reduced risk of selenium toxicity and as a potential chemopreventive agent **[4].** The role of seleno compounds as chemopreventive and chemotherapeutic agents has been supported by a large number of epidemiological, preclinical and clinical studies **[5].**

Fermented wheat germ extract (FWGE) is a concentrated extract of wheat germ derived from the germ of the wheat plant and differs from ordinary wheat germ in that it is fermented with yeast to concentrate biologically-active benzoquinones. It contains two quinones, 2-methoxy benzoquinone and 2,6-dimethoxybenzquinone that likely play a significant role in exerting several of its biological properties **[6].** Preclinical in vitro and in vivo data suggested antiproliferative, antimetastatic and immunological effects of FWGE **[7, 8].** FWGE is not a drug, nor an alternative to standard anticancer drugs or standard therapies: FWGE is a dietary supplement to be given to cancer patients to help drugs to work better **[9].**

MATERIALS AND METHODS

Animals

Female Swiss albino mice weighing 20–25 g were obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA, Giza, Egypt). Animals were kept under standard conditions and were allowed free access to a standard requirement diet and water *ad.* Libitum. Animals were kept under a controlled lighting condition (light: dark, 12 h:12 h).

Ehrlich Ascites Carcinoma Cells:

A line of Ehrlich Ascites Carcinoma (EAC) cells was supplied from National Cancer Institute, Cancer Biology Department.Egypt.

Tumor induction:

Solid tumors were produced by intramuscular inoculation with 0.2 ml of EAC, which contained 2.5 x 10^6 viable EAC cells, in the right thigh of the lower limb of each mouse. Mice with a palpable solid tumor, its diameter was 10mm³, that developed within 10 days after inoculation were used in this study.

Chemicals:

Wheat germ was obtained from local market and stored in sealed plastic bags at 4^{o}C before use. Selenium and all chemical and kits purchased from Sigma (USA).

Production of FWGE and FWGE-nano-Se mixture for bioevaluation of antitumor activity as anticancer was prepared as follows:

Preparation of FWGE:

Thirty grams of active *Saccharomyces cerevisiae* cells were suspended in 270ml dist. water and mixed with 90g of wheat germ.The mixture was then fermented at 37ºC for 48hs in incubator. The suspension was centrifuged at 3000rpm for 10min and the supernatant was freeze dried by (LyoTrap (NCRRT) USA) and the resulted powder was kept in sealed vial.

Preparation of FWGE-nano-Se mixture:

To 100ml deionized water add 1 ml selenious acid (0.04mM), 4ml of 0.2mM GSH solution containing 200mg of bovine serum albumin with stirring to initiate the reaction.

The pH of the mixture was adjusted to 7.2 with 1.0 M sodium hydroxide, during which the red elemental Se and oxidized glutathione (GSSG) formed. The reaction lasted 1hour under sonication. The red solution was dialyzed against doubly distilled water for 96 h with the water changing every 24 h to separate GSSG from Nano-Se. Centrifugation at 20000rpm(Hettich cooling centrifug; type Werk Nr. Made in Germany).The pellets were mixed with fermented wheat germ extract under sonication conditions for 1 hour to form mixture.

Cell viability assay:

EAC viable cells were counted by *trypan blue exclusion* method where, 10µl trypan blue (0.05%) was mixed with 10µl of the cell suspensions. Within 5 minutes, the mixture was spread onto haemocytometer, covered with a cover slip and then cells were examined under microscope. Dead cells are blue stained but viable cells are not **[10].**

Experimental design:

Sixty female Swiss albino mice were divided into 4 groups each contain 15 mice as follows:

Group (1): Served as negative control and orally received saline served as negative control group (NTBM: Non-tumor bearing mice). **Group (2):** Tumor bearing mice without any treatment served as positive control group (TBM) for 6 weeks.**Group (3):** Tumor bearing mice received FWGE at dose of 3gm /Kg body weight/day ($TBM_{(FWGE)}$) for 6weeks. **Group (4):** Tumor bearing mice received FWGE-nanoSe at a dose of 2.125 g (dry weight)/kg body weight/ day for FWGE and 2 mg/kg body weight for nano-Se ($TBM_{(FWGE\text{-}nanoSe)}$) for 6 weeks.

Blood and tissue sampling:

Directly, after animals were sacrificed, blood was collected after 2 and 6 weeks. liver and tumor were dissected out every 2, and 6 weeks from the beginning of treatment, part of them was homogenated and samples (N.B. muscle tissue of negative control group was dissected to be compared with tumor bearing group) were prepared in ice-cold phosphate buffer which used for determination of antioxidant parameters and the other portions of tumor and liver at the end of experiment (after 6 weeks of treatments) were dissected and kept in 10% formalin for histopathological examination and apoptosis detection (in tumor tissue).

Tumor volume determination

After 10 days from inoculation of Ehrlich carcinoma, tumor volume was measured twice a week using a Vernier caliper and determined by applying the following equation according to **Jensen *et al.* [11]:** $$\textit{Tumor volume} = 1/2(\textit{length} \times \textit{width}^2)$$

Where length is the greatest longitudinal diameter and width is the greatest transverse diameter.

Estimation of Malondialdhyde (MDA) level:

Lipid peroxidation is measured colorimetrically according to the method of **Yoshioka *et al*. [12]** based on measurement of Malondialdhyde (MDA) as one of the main end products of lipid peroxidation by thiobarbituric acid test.

Estimation of catalase (CAT) activity

Catalase activity was measured in plasma and 10% liver homogenate according to the method of **Sinha [13].** The dichromate/ acetic acid reagent can be thought of as a "stop bath" for catalase activity. As soon as enzyme reaction mixture hits the acetic acid, its activity is inhibited, any hydrogen peroxide, which has not been split by catalase will react with dichromate to give a blue precipitate of perchromic acid. This unstable precipitate was then decomposed by heating to give the green color solution which was measured spectrophotometery at 570 nm.

Estimation of glutathione content (GSH):

Glutathione was measured according to the colorimetric method of **Beutler *et al*. [14].** This method is based on spectrophotometrically measurement of the yellow color of 2-nitro-5-thiobenzoic acid which was produced as one product of this reaction:

Glutathione + 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) ⟶ 2-nitro-5-thiobenzoic acid + glutathione disulfide (GSSG).

Estimation of superoxide dismutase activity (SOD):

SOD activity is measured in blood and 10% tissue homogenate according to the method of **Minami & Yoshikawa [15]**.SOD catalyzes the dismutation of the superoxide radical (O^-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2).

$$4O^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Superoxide ions, generated from auto-oxidation of pyrogallol, convert the nitro blue tetrazolium chloride (NBT) to NBT-diformazan which absorbs light at 550 nm.

SOD reduces the superoxide ion concentration thereby lowering the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity present in samples.

Estimation of nitrate/nitrite ($NO_{(x)}$):

Nitric oxide was determined according to the method described by **Miranda *et al.* [16].** Nitric oxide is relatively unstable in the presences of molecular oxygen**,** with an apparent half life approximately 3-5 seconds and is rapidly oxidized to nitrate and nitrite totally designated as NO_x. A high correlation between endogenous nitric oxide production and nitrite/nitrate ($NO_{x)}$ levels has been established. The measurement of these levels provides a reliable and quantitative estimate of nitric oxide output in vivo.The assay determines total nitrite/nitrate level based on the reduction of any nitrate to nitrite by vanadium followed by the detection of total nitrite by Griess reagent. The Griess reaction entails formation of a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with bicyclic amines such as N-(1-naphthyl) ethylendiamine. The chromophoric azo derivative can be measured colorimetric ally at 540 nm.

Estimation of Glutathione Peroxidase(GPx):

GPx is determined by using the method of **Gross *et al.* [17]** and **Necheles *et al*.[18].** The method is a linked enzyme reaction in which the oxidized glutathione (GSSG) formed by the action of H_2O_2 and GSH-px, is converted back to its reduced form in the presence of glutathione reductase (GSSG-R) and NADPH. The GSH is thus maintained at a constant concentration and the reaction is followed by measuring the stoichiometric oxidation of NADPH. In this method the amount of residual GSH left after exposure to enzyme activity for a fixed time is measured calorimetrically.

Liver function tests:

Estimation of aspartate aminotransferase (AST) activity:

AST activity in plasma was determined by a colorimetric method as described by **Reitman and Frankel [19]** using a diagnostic kit supplied by (**Plasmatek, Germany)**.The enzyme AST catalyzes the following reaction:

L-aspartate + α-Ketoglutarate $\xrightarrow{\text{AST}}$ oxalacetate + L-glutamate

The formed oxalacetate reacts with 2,4-dinitrophenylhydrazine to form oxalacetate hydrazones, which are brown in alkaline medium. The product is determined spectrophotometrically at λ 546 nm.

Estimation of alanine aminotransferase (ALT) activity:

ALT activity in plasma was determined by a colorimetric method as described by **Reitman and Frankel [19]** using a diagnostic kit supplied by (**plasmatek, Germany)**.The enzyme ALT catalyzes the following reaction:

L-alanine + α-Ketoglutarate $\xrightarrow{\text{ALT}}$ pyruvate + L-glutamate

The formed pyruvate reacts with 2,4-dinitrophenylhydrazine to form pyruvate hydrazones, which are brown in alkaline medium. The product is determined spectrophotometrically at 505 nm.

Estimation of gamma glutamyl transferase (GGT) activity:

Plasma gamma-glutamyl-transferase was determined according to **Szasz [20]** using a diagnostic kit supplied by (**Pointe Scientific, INC Co., USA).** Gamma-Glutamyl is transferred from gamma-glutamyl-3-carboxy-4-nitroanilide to glycylglycine by gamma-glutamyl-transferase. The m-carboxy-p-nitroaniline formed was measured kinetically at 405 nm.

Kidney function tests

Estimation of creatinine in plasma

Creatinine in plasma was determined by a colorimetric method as described by **Henry *et al*. [21]** using a diagnostic kit supplied by **(Diamond ,Egypt)**. Creatinine in alkaline solution reacts with picrate to form a colored complex.

Estimation of urea in plasma:

Urea in plasma was determined by an enzymatic colorimetric method as described by **Palton and Crouch [22]** using a diagnostic kit supplied by (**Diamond, Egypt). (*Urease – modified Berthelot reaction)*** En ermination of urea is according to the following reaction:

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

In an alkaline media , the ammonium ions react with the salicylate and hypochlorite to form a green colored indophenol.

Characterization of cell death within tumor tissue (Apoptosis):

Apoptosis was determined using Acridine Orange - Ethidium Bromide Staining **[23].** Acridine orange and ethidium bromide are fluorescent DNA intercalating dyes. Viable cells are acridine orange permeable and ethidium bromide impermeable. Healthy and early stage apoptotic cells take up acridine orange and fluoresce green. Apoptotic cells take up ethidium bromide dye and fluoresce orange.

Histopathological examination:

Specimens from tumor and liver were fixed in 10% buffered neutral formalin solution, dehydrated, embedded in paraffin and then five-micron thick paraffin sections were prepared. Slides were then stained with hematoxylin and eosin "H&E" by routine procedure.

Statistical analysis

Statistical analysis was done using SPSS software version 15. The inter-group variation was measured by one way analysis of variance (ANOVA) followed by Post Hoc LSD test. Results were expressed as mean ± SE. The mean difference is significant at the 0.05 level.

Results:

***In vitro* studies:**

Cytotoxicity of fermented wheat germ extract (FWGE) and fermented wheat germ extract-nano-Se (FWGE-nano-Se) mixture against EAC cells:

Treatment of EAC cells with different concentrations of FWGE (0.21-85 mg/ml) for one hour showed cytotoxicity with 50% inhibition of cell survival (IC50) at concentration of 0.8mg /ml , Table (1). While, in case FWGE-nano-Se, treatment of EAC cells with (FWGE at different concentrations (0.21-85 mg/ml) +nano-Se at concentrations (0.2-80 µg /ml)) for one hour showed cytotoxicity with IC_{50} at concentration of 0.8 mg /ml FWGE +0.75 µg /ml nano-Se using trypan blue exclusion method, table(2).

Table 1. Surviving percent in EAC cells as affected by different concentrations of FWGE after 1 hour incubation:

FWGE concentration (mg /ml)	Cell survival % using trypan blue exclusion method
0	98.6±0.73
0.21	96.5±1.46
0.425	85.68±1.77
0.85	20.48±2.03
8.5	11.68±1.61
85	1.78±1.01

Data are expressed as mean ±SE.

Table 2. Surviving percent in EAC cells as affected by different concentrations of FWGE-nano-Se mixture after 1 hour incubation:

FWGE-nano-Se mixture concentration		Cell survival % using trypan blue exclusion method
FWGE(mg /ml)	nano-Se(µg /ml)	
0	0	98.60 ±0.7
0.21	0.2	95.40±1.2
0.425	0.4	83.40±1.8
0.85	0.8	26.80±6.8
8.5	8	8.80±2.4
85	80	1.22±0.8

Data are expressed as mean ±SE.

***In vivo* studies:**

Tumor volume:

Tumor volume (mm^3) in Fig.(1) of positive control group (TBM) was progressively increased in its size reached more than ten times its initial volume at the end of the experimental period. While, tumor volume was significantly decreased in FWGE and FWGE-nano-Se mixture treated groups compared to untreated group (TBM) and continued till the end of the experiment. FWGEand FWGE-nano-Se mixture treated groups possessed 52% and 61% reduction in tumor volume respectively.

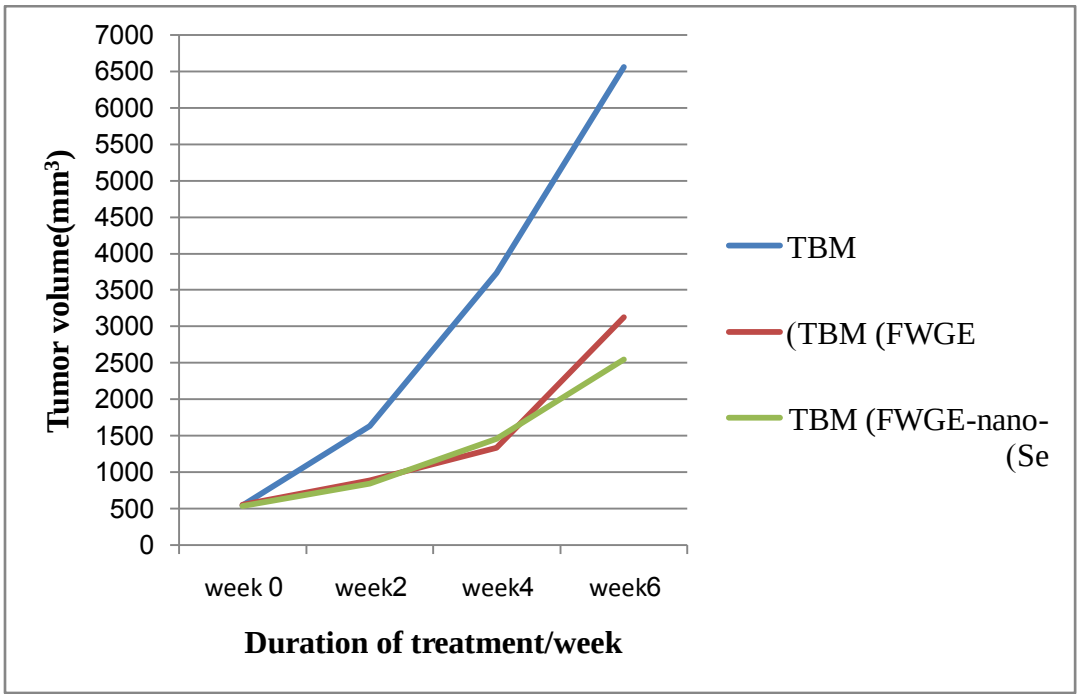

Figure 1 Effect of FWGE and FWGE-nano-Se mixture on tumor volume of Ehrlich solid tumor.

Antioxidant Effect of FWGE and FWGE-nano-Se mixture:

a) Effect on the activity of SOD:

From table 3, SOD activity ***in blood:*** was highly significant decreased in TBM ($P < 0.01$) after 2 and 6 weeks compared to NTBM. While it was significantly increased ($P < 0.05$) after 6 weeks in TBM *(FWGE)* compared to TBM. Moreover, it was significantly increased after 2 and6 weeks in TBM *(FWGE-nano-Se)* compared to TBM and TBM *(FWGE).* ***In liver tissue:*** the data revealed very highly significant decrease in TBM ($P < 0.001$) after 2 weeks compared to NTBM. But it revealed significant increase ($P < 0.05$) after 2 and 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM. ***In tumor tissue:*** results showed very highly significant decrease after 2 and 6weeks in TBM group compared to NTBM group and significant increase after 2 and 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM. Furthermore, it was significantly increased after 6 weeks in TBM *(FWGE-nano-Se)* compared to TBM *(FWGE).*

Table 3: Effect of FWGE and FWGE-nano-Se mixture on SOD activity in blood, liver and tumor tissue:

Each value is the mean ± SEM. Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01; very highly significant: ***p<0.001 from NTBM. a, significant from TBM p<0.05. b, significant from TBM $_{(FWGE)}$group p<0.05. c, significant from TBM$_{(FWGE\text{-}nano\text{-}Se)}$group p<0.05.

Parameter / Groups	SOD activity					
	Blood (U /ml)		Liver Tissue (U / g Tissue)		Tumor Tissue (U / g Tissue)	
	2 Weeks	6 Weeks	2 Weeks	6 Weeks	2 Weeks	6 Weeks
NTBM	3.34 ± 0.09	3.11 ± 0.13	4.57 ± 0.24	5.16 ± 0.13	4.86 ± 0.096	7.64±0.1
TBM	2.58± 0.15**	2.36± 0.26**	3.72 ± 0.14***	4.42 ± 0.18	4.08 ± 0.12***	6.46 ± 0.17***
TBM *(FWGE)*	2.74 ± 0.15 [c]	3.01 ± 0.25 [a c]	4.45 ± 0.15 [a]	6.67 ± 0.300 [a]	4.66 ± 0.18 [a]	7.57 ± 0.25 [a c]
TBM$_{(FWGE\text{-}nano\text{-}Se)}$	3.92 ± 0.30 [a b]	4.04 ± 0.11 [a b]	4.65 ± 0.09 [a]	6.84 ± 0.51 [a]	4.76 ± 0.14 [a]	8.11 ± 0.06 [a b]

b) Effect on the activity of GPx:

As indicated in table 4, GPx activity ***in blood:*** was very highly significant decreased in TBM after 2 and 6 weeks compared to NTBM. While it was significantly increased after 6 weeks in TBM *(FWGE)* compared to TBM. Moreover, it was significantly increased in TBM *(FWGE-nano-Se)* after 2 and 6 weeks compared to TBM. Furthermore, it was significantly increased in TBM *(FWGE-nano-Se)* after 2 weeks compared to TBM *(FWGE)* . ***In liver tissue:*** GPx activity was highly significant decreased in TBM after 6 weeks compared to NTBM. ***In tumor tissue:*** GPx activity was very highly significant decreased after 2and 6weeks in TBM group compared to NTBM group but it was significantly increased after 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM. Furthermore, it was significantly increased in TBM *(FWGE-nano-Se)* after 6 weeks compared to TBM *(FWGE).*

Table 4. Effect of FWGE and FWGE-nano-Se mixture on GPx activity in blood, liver and tumor tissue:

Parameter / Groups	GPx activity					
	Blood (Consumed reduced glutathione /min /ml)		Liver Tissue (Consumed reduced glutathione /min /g)		Tumor Tissue (Consumed reduced glutathione /min /g)	
	2 Weeks	6 Weeks	2 Weeks	6 Weeks	2 Weeks	6 Weeks
NTBM	0.78 ± 0.005	0.78 ± 0.011	0.61 ± 0.005	0.64 ± 0.008	0.517±0.005	0.7±0.001
TBM	0.64 ± 0.007***	0.66 ± 0.010***	0.49 ± 0.070	0.58 ± 0.005**	0.46 ± 0.008***	0.56 ± 0.008***
TBM *(FWGE)*	0.66 ± 0.012 [c]	0.79 ± 0.005 [a]	0.56 ± 0.039	0.59 ± 0.002	0.50 ± 0.049	0.64 ± 0.006 [a c]
TBM *(FWGE-nano-Se)*	0.69 ± 0.010 [a b]	0.80 ± 0.011 [a]	0.45 ± 0.021	0.61 ± 0.003	0.56 ± 0.013	0.72 ± 0.006 [a b]

Each value is the mean ± SEM. Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01; very highly significant: ***p<0.001 from NTBM. a, significant from TBM p<0.05. b, significant from TBM $_{(FWGE)}$group p<0.05. c, significant from TBM$_{(FWGE\text{-}nano\text{-}Se)}$group p<0.05.

c) Effect on GSH content:

From table 5, GSH content ***in blood:*** TBM group showed very highly significant decrease in GSH content after 2 and 6 weeks compared to NTBM. While, TBM *(FWGE)* and TBM *(FWGE-nano-Se)* showed significant increase after 2and 6 weeks compared to TBM. Moreover, it was significantly increased in TBM *(FWGE-nano-Se)* after 6 weeks compared to TBM *(FWGE).* ***In liver tissue:*** GSH content was very highly significant decreased in TBM after2 and 6 weeks compared to NTBM. But, it was significantly increased after 2 and 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM. ***In tumor tissue:*** GSH content in TBM showed highly significant decrease after2 and 6 weeks compared to NTBM and it was significantly increased after 6 weeks in TBM *(FWGE)* compared to TBM. Furthermore, it was significantly decreased in TBM *(FWGE-nano-Se)* after 6 weeks compared to TBM *(FWGE).*

Table 5. Effect of FWGE and FWGE-nano-Se mixture on GSH content in blood, liver and tumor tissue:

Parameter Groups	GSH content					
	Blood (mg/dl)		Liver Tissue (mg / g Tissue)		Tumor Tissue (mg / g Tissue)	
	2 Weeks	6 Weeks	2 Weeks	6 Weeks	2 Weeks	6 Weeks
NTBM	137.74 ± 1.64	141.16 ± 5.26	112.21 ± 0.90	164.90 ± 5.79	113.45±3.2	157.25±3.8
TBM	109.96 ± 2.13***	112.29 ± 3.335***	71.00 ± 1.43***	135.41 ± 4.62***	99.02 ± 1.85**	144.72 ± 1.45**
TBM *(FWGE)*	153.42 ± 2.73 [a]	141.77 ± 0.93 [a c]	103.91 ± 4.23 [a]	160.79 ± 2.92 [a]	109.88 ± 2.35	165.21 ± 2.199 [a c]
TBM *(FWGE-nano-Se)*	156.687 ± 3.23 [a]	162.19 ± 1.14 [a b]	105.30 ± 3.14 [a]	166.69 ± 1.80 [a]	109.81 ± 1.88	149.23 ± 2.36 [b]

Each value is the mean ± SEM. Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01; very highly significant: ***p<0.001 from NTBM. a, significant from TBM p<0.05. b, significant from TBM $_{(FWGE)}$group p<0.05. c, significant from TBM$_{(FWGE\text{-}nano\text{-}Se)}$group p<0.05.

d) Effect on the activity of CAT:

As shown in table (6) catalase activity ***in blood:*** was very highly significant decreased in TBM after 2 and 6 weeks compared to NTBM. While, it was significantly increased after 2 and6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM. ***In liver tissue:*** CAT Activity was very highly significant decreased in TBM after 6 weeks compared to NTBM. But it was significantly increased after 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM. Furthermore, it was significantly increased in TBM *(FWGE-nano-Se)* after 6 weeks compared to TBM *(FWGE).* ***In tumor tissue:*** data showed that CAT Activity was very highly significant increase in TBM After 2 weeks, became highly significant decrease after6 weeks compared to NTBM and it was was significantly increased after 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM.

Table 6. Effect of FWGE and FWGE-nano-Se mixture on catalase activity in blood, liver and tumor tissue:

Each value is the mean ± SEM. Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01; very highly significant: ***p<0.001 from NTBM. a, significant from TBM p<0.05. b, significant from TBM $_{(FWGE)}$group p<0.05. c, significant from TBM$_{(FWGE\text{-}nano\text{-}Se)}$group p<0.05.

Parameter / Groups	CAT Activity					
	Blood (U/L)		Liver Tissue (U/g tissue)		Tumor Tissue (U/g tissue)	
	2 Weeks	6 Weeks	2 Weeks	6 Weeks	2 Weeks	6 Weeks
NTBM	0.595 ± 0.014	0.633 ± 0.006	0.154 ± 0.003	0.160 ± 0.005	0.15± 0.003	0.12± 0.002
TBM	0.350± 0.017***	0.503 ± 0.009***	0.164 ± 0.004	0.101 ± 0.006***	0.167 ± 0.003***	0.094 ± 0.003**
TBM *(FWGE)*	0.445 ± 0.019 [a]	0.608 ± 0.006 [a]	0.165 ± 0.003	0.124 ± 0.003 [ac]	0.165 ± 0.003	0.121 ± 0.011 [a]
TBM *(FWGE-nano-Se)*	0.450 ± 0.018 [a]	0.596 ± 0.020 [a]	0.173 ± 0.005	0.156 ± 0.008 [a b]	0.173 ± 0.01	0.126 ± 0.006 [a]

e) Effect on MDA concentration:

From table 7, **MDA concentration *in blood:*** TBM group showed very highly significant increase in MDA concentration after 2 and 6 weeks compared to NTBM. While, TBM *(FWGE)* showed significant decrease after 6 weeks but TBM *(FWGE-nano-Se)* showed significant decrease after 2 and 6 weeks compared to TBM. Moreover, it was significantly decreased in TBM *(FWGE-nano-Se)* after 2 weeks compared to TBM *(FWGE).* ***In liver tissue:*** MDA concentration was significantly increased in TBM after 6 weeks compared to NTBM. But, it was significantly decreased after 6 weeks in TBM *(FWGE-nano-Se)* compared to TBM. ***In tumor tissue:*** TBM showed highly significant increase after 2 weeks became very highly significant increase after 6 weeks compared to NTBM and it was significantly decreased after 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM.

Table 7: Effect of FWGE and FWGE-nano-Se mixture on MDA concentration in blood, liver and tumor tissue:

Parameter / Groups	MDA concentration					
	Blood(µM/ml)		Liver Tissue (µM/g Tissue)		Tumor Tissue (µM/g Tissue)	
	2 Weeks	6 Weeks	2 Weeks	6 Weeks	2 Weeks	6 Weeks
NTBM	91.74 ± 1.68	90.74 ± 1.66	210.8 ± 7.9	215.48 ± 7.35	174.5±5.8	185±2.7
TBM	137.73 ± 4.88***	111.66± 2.3***	228.48 ± 9.09	254.14 ± 15.24[*]	219.97 ± 8.04**	241.81 ± 6.87***
TBM *(FWGE)*	137.66 ± 6.7 [c]	100.82 ± 2.46 [a]	236.97 ± 16.74	225.65 ± 11.24	221.98± 7.994	190.82± 6.6 [a]
TBM *(FWGE-nano-Se)*	118.99 ± 4.4 [a b]	98.91 ± 2.14 [a]	236.98 ± 8.48	203.15 ± 16.35[a]	234.14 ± 12.68	202.65 ± 12.66 [a]

Each value is the mean ± SEM. Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01; very highly significant: ***p<0.001 from NTBM. a, significant from TBM p<0.05. b, significant from TBM $_{(FWGE)}$group p<0.05. c, significant from TBM$_{(FWGE\text{-}nano\text{-}Se)}$group p<0.05.

f) Effect on $NO_{(X)}$ concentration:

Table 8 ***showed*** that **$NO_{(X)}$ concentration *in blood:*** was very highly significant increased in TBM after 2 and 6 weeks compared to NTBM. While it was significantly decreased after 2 and 6 weeks in TBM *(FWGE)* and after 6 weeks in TBM *(FWGE-nano-Se)* compared to TBM. ***In liver tissue:*** **$NO_{(X)}$** concentration was very highly significant increased in TBM after 2 and 6 weeks compared to NTBM. But, it was significantly decreased after 2 and 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM. ***In tumor tissue***, it was highly significant increase after 2 weeks became very highly significant increase after 6 weeks in TBM compared to NTBM and it was significantly decreased after 6 weeks in TBM *(FWGE)* and TBM *(FWGE-nano-Se)* compared to TBM.

Table 8: Effect of FWGE and FWGE-nano-Se mixture on $NO_{(X)}$ concentration in blood, liver and tumor tissue:

Parameter Groups	$NO_{(X)}$ concentration					
	Blood (µM/L)		Liver Tissue (µM/g tissue)		Tumor Tissue (µM/g tissue)	
	2 Weeks	6 Weeks	2 Weeks	6 Weeks	2 Weeks	6 Weeks
NTBM	20.96 ± 0.77	20.9 ± 0.43	21.78 ± 0.81	21.91± 0.562	14.2±0.44	12.57±0.11
TBM	31.5 ± 0.82***	35.4 ± 0.64***	28.52± 1.42***	31.49 ± 0.63***	18.60 ± 0.84**	23.27 ± 1.73***
TBM *(FWGE)*	28.2 ± 0.64 [a]	20.12 ± 0.78 [a]	25.05 ± 0.38 [a]	23.25 ± 0.72 [a]	17.84 ± 0.578	14.29 ± 0.45 [a]
TBM *(FWGE-nano-Se)*	30.18 ± 0.39	19.3 ± 0.52 [a]	25.68 ± 1.01 [a]	21.52 ± 0.75 [a]	16.27 ± 0.78	13.63 ± 0.73 [a]

Each value is the mean ± SEM. Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01; very highly significant: ***p<0.001 from NTBM. a, significant from TBM p<0.05. b, significant from TBM $_{(FWGE)}$group p<0.05. c, significant from TBM$_{(FWGE\text{-}nano\text{-}Se)}$group p<0.05.

Liver and kidney function tests:

From table (9), in TBM group, AST activity was very highly significant increased after2 weeks became significantly decreased after6 weeks and GGT activity was very highly significant increased after2 and 6 weeks compared to NTBM group. Treatment with FWGE showed significant decrease after 6 weeks in ALT, AST, urea and creatinine but, after 2 and 6 weeks in GGT activity compared to TBM group. While, treatment with FWGE-nano-Se showed significant decrease in urea and creatinine concentration after 6 weeks compared to TBM.

Table 9 Changes in blood ALT, AST, GGT, urea and creatinine in controls, FWGE and FWGE-nano-Se mixture treated mice groups.

Parameters group	ALT(U/L)		AST(U/L)		GGT(U/L)		Urea(mg/dl)		Creatinine(mg/dl)	
	2wks	6wks	2wks	6wks	2wks	6wks	2wks	6wks	2wks	6wks
NTBM	50.5 ± 2.8	58.3 ± 5.51	96.5 ± 4.99	98.6 ± 1.15	31.01 ± 2.2	33.7 ± 2.1	29.65 ± 2.5	30.6 ± 0.64	0.79± 0.07	0.82± .017
TBM	56.5 ± 4.8	73.2 ± 9.7	130.4± 6.2***	122.± 3.02*	52.6 ± 0.7***	65.75 ± 4.8***	28.85 ± 2.8	34.2 ± 1.65	0.77± 0.08	0.91± .044
TBM *(FWGE)*	62.1 ± 8.8	52.9 ± 3.1 [a]	119.29 ± 2.4	102.6 [a] ±10.6	46.97 ± 1.8 [a]	41.4± 1.5 [a]	30.9 ± 2.46	29.5 ± 1.58 [a]	0.82± 0.06	0.79± 0.04 [a]
TBM *(FWGE-nano-Se)*	53.2 ± 0.5	55.2 ± 2.6	112.49 ± 7.75	104.3 ± 2.4	48.58 ± 1.65	38.58 ± 1.3 [a]	27.85 ± 1.0	28.55 ± 0.5 [a]	0.75± 0.03	0.76± 0.01 [a]

Each value is the mean ± SEM. Non-significant (N.S): $p>0.05$; Significant: *$p<0.05$; highly significant: ** $p<0.01$; very highly significant: ***$p<0.001$ from NTBM. a, significant from TBM $p<0.05$. b, significant from $TBM_{(FWGE)}$group $p<0.05$. c, significant from $TBM_{(FWGE\text{-}nano\text{-}Se)}$group $p<0.05$.

Histopathological examination:

In liver tissue:

Histopathological examination of **liver** sections of positive control group showed numerous neoplastic foci widely spreaded all over the liver in comparison to the control liver (normal liver) which either distributed as a focal mass (Fig.1a) or distributed mainly among the hepatic cords (Fig.2b). Liver of tumor bearing mice treated with FWGE showed distributed tumor cells among the hepatic cells (Fig.2c). While, the liver of tumor bearing mice treated with FWGE-nano-Se mixture showed distribution of neoplastic cells among the hepatic cords (Fig2d).

In muscle tissue:

Histopathological examination of **muscle sections** of positive control group showed extensive invasion of neoplastic cell and necrosis of muscular tissue (Fig 3a). But in $TBM_{(FWGE)}$ showing focal aggregation of neoplastic cells surrounded by muscular tissue capsule (Fig.3b). While, in $TBM_{(FWGE\text{-}nano\text{-}Se)}$ showing small aggregated foci of neoplastic cells (Fig.3c).

Characterization of cell death within the tumor (Apoptosis):

Analysis of our photographs revealed normal mice tumor cells stained green with the presence of some level of apoptotic cells as expected (stained orange) presented in figure (4a,b).Also, analysis of the results revealed induction of apoptosis under the effect of FWGE treatment with the presence of bright green cells in the middle area of section in Fig.(4c,d). Also, Fig.(4 e,f) revealed that combination of FWGE with Se nanoparticles reply more antitumor efficacy by induction of more apoptosis.

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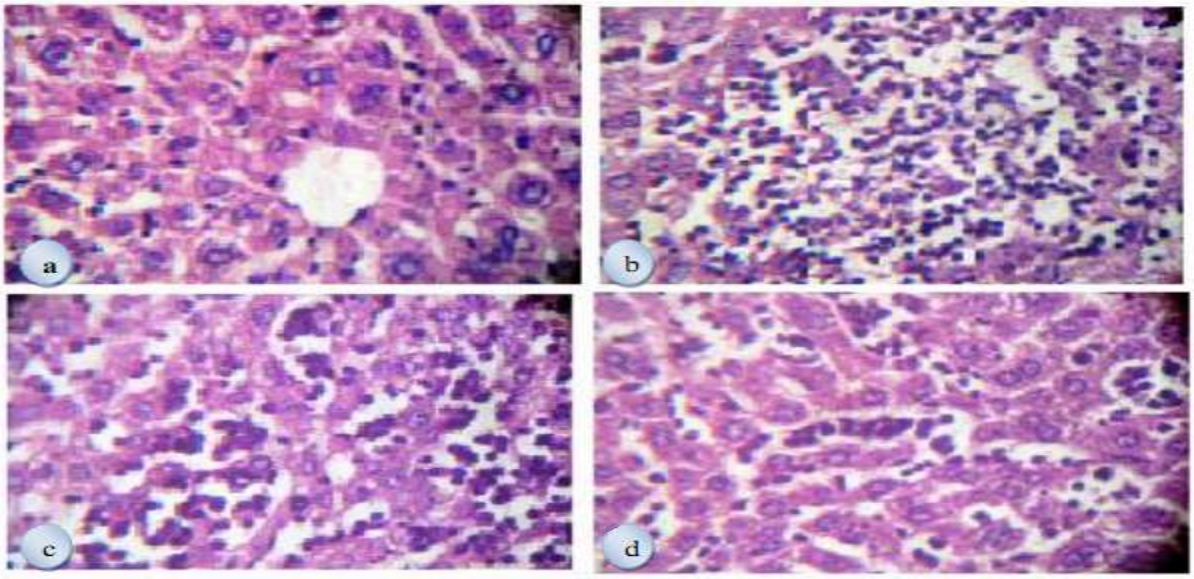

Fig 2: Photomicrographs of sections in liver stained by H& E (a) Liver of negative control group showing normal hepatic architecture. (b) Liver of mice bearing Ehrlich carcinoma (positive control group) showing focal aggregation of neoplastic cells. (c) Liver of tumor bearing mice treated with FWGE showing distributed neoplastic cells among the hepatic cells. (d) Liver of tumor bearing mice treated with FWGE + nano-Se showing neoplastic cells among the hepatic cords (H&E x 1200).

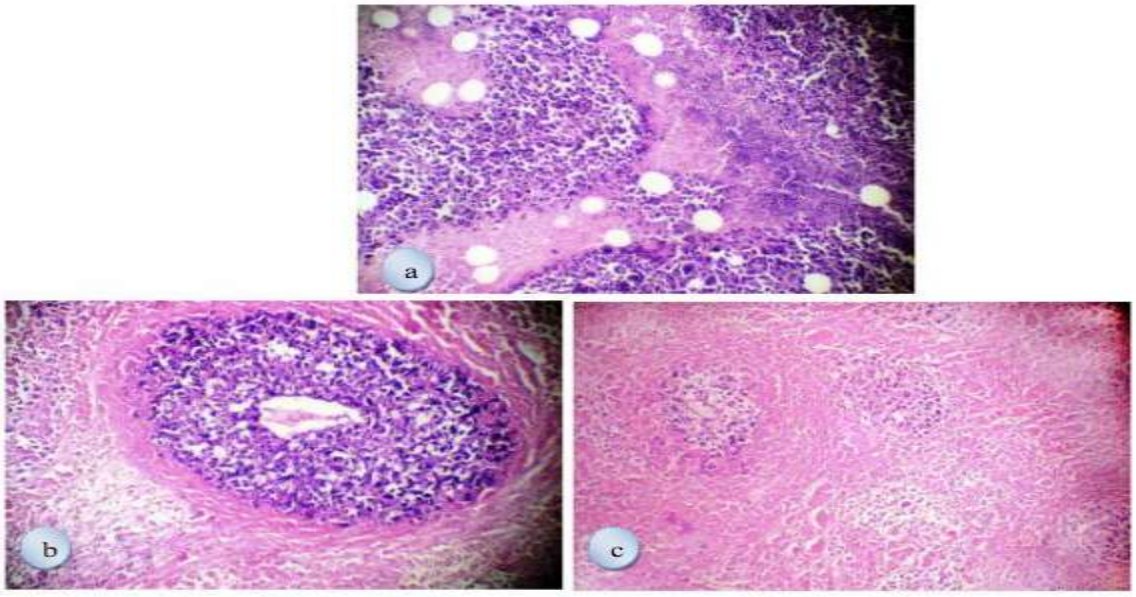

Fig 3: Photomicrographs of sections in Ehrlich solid tumor (EST) stained by H&E. (a) Thigh muscle of mice bearing Ehrlich carcinoma(positive control group) showing extensive invasion of neoplastic cells(H&E x 300). (b) Thigh muscle of tumor bearing mice treated with FWGE showing focal aggregation of neoplastic cells surrounded by muscular tissue capsule (H&E x 300). (c) Thigh muscle of tumor bearing mice treated with FWGE - nano-Se showing small aggregated foci of neoplastic cells (H&E x 150).

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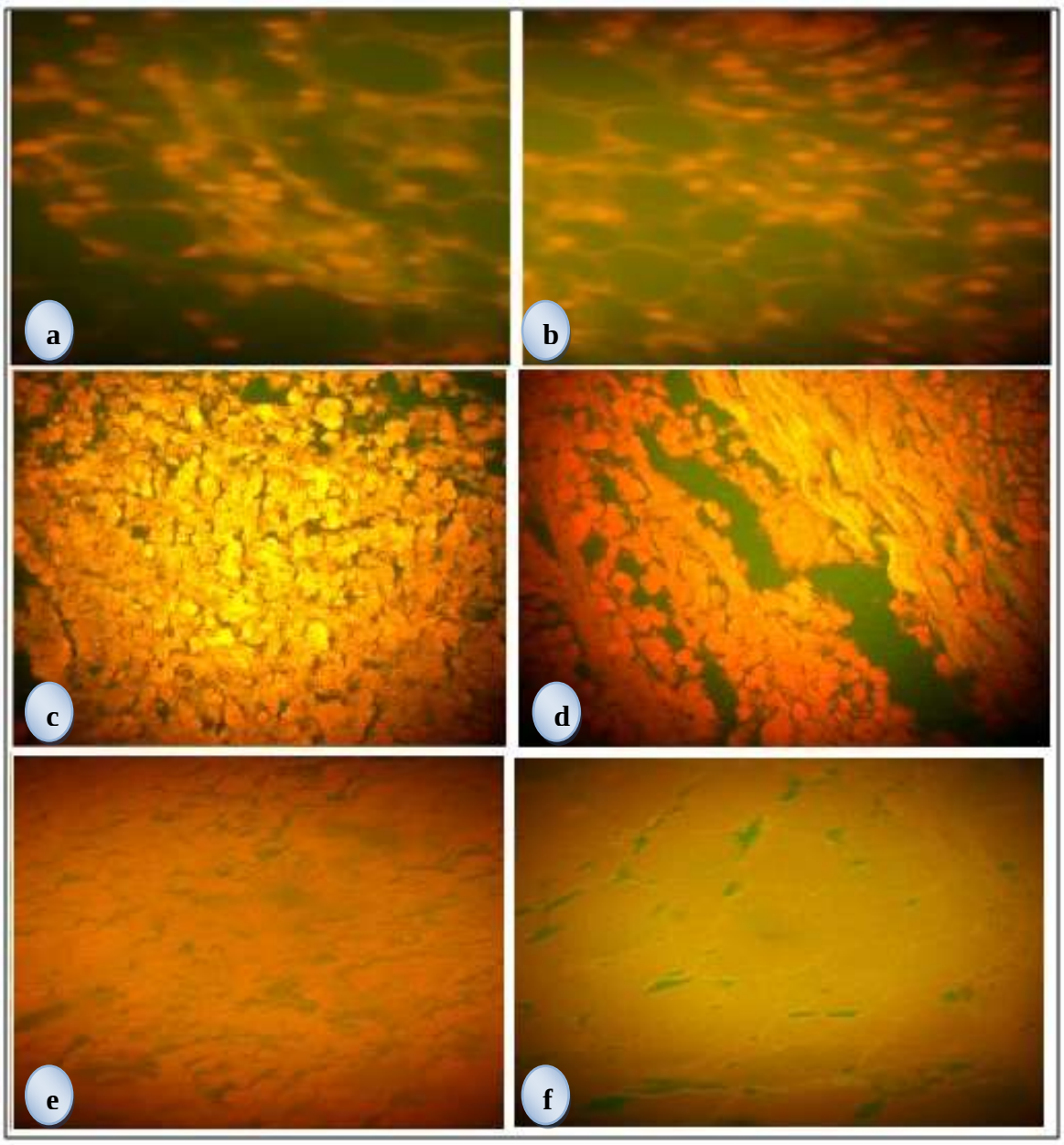

Figure 4 Acridin Orange (AO)/Ethedium Bromide (EB) staining sections (a,b) of mice control tumors.(c,d) Sections in tumors of FWGE treated mice. (e,f) Sections in tumors of FWGE-nano-Se treated mice (AO/EB, X 40).

Discussion:

Application of FWGE and FWGE-nano-Se mixture on EAC cells table (1&2) showed cytotoxicity with maximum cell mortality (98.22% and 98.78%)respectively at 85mg/ml FWGE and 80 µg /ml nano-Se after 1 hour incubation. Our results are in agreement with **Tomoskozi-Farkas & Daood [24]** who stated that, some benzoquinones such as 2,6-dimehoxy benzoquinone (2,6-DMBQ) have been proved to exhibit cytotoxic effect in EATC, and thereby inhibit tumor propagation and metastases. Also, **Hedvegi *et al.* [25]** reported that 2,6-DMBQ and

2-MBQ (benzoquinones presents in FWGE) are cytotoxic for malignant tumor cells. FWGE induced apoptosis and exerted significant antiproliferative activity in a broad spectrum of tumor cell lines.
FWGE and FWGE -nanoSe mixture fig (1) showed marked regression in tumor growth that were observed by the significant reduction in tumor volume and tumor weight when compared with untreated group. These observations are in agreement with those recorded by **Hidvegi *et al.* [26]** who concluded that, growth inhibition of EAC tumor can be achieved by treatment of tumor bearing mice with a mixture of 2,6-DMBQ and ascorbic acid.
One of the largest known natural source for 2,6-dimethoxy-benzoquinone (DMBQ) and 2-methoxy-benzoquinone is wheat germ as glycosides; yeast glycosidase activity present during fermentation leads to release of the benzoquinones as aglycones **[6].** The biological activity of quinine is connected with their participation in redoxy-cycles in the form of free reactive radicals. Their ability to produce aryl-nucleophil compounds, particularly by reaction with thiol and amino groups may explain the extreme activity of these compounds **[26].**

Results of antioxidant parameters of TBM group (significant increase in MDA and $NO_{(X)}$ and significant decrease in SOD, GPx, GSH and CAT) are in agreement with **Kumaraguruparan *et al.* [27]** who found that the presence of tumor caused disequilibria of the antioxidant defense system. Moreover, **Hayat [28]** demonstrated that, lipid peroxidation level was significantly increased in blood, liver and tumor tissues of EAC mice when compared with control group. In contrary, **Cheeseman *et al.* [29]** who suggested that, there is a decrease rate of lipid peroxidation in liver tumor cell than normal liver cells.
Also, our findings are in agreement with **Saygili *et al.* [30]** who demonstrated that a decrease in blood GSH in circulation has been reported in several diseases including malignancies.
Decline in SOD activity recorded in mice bearing Ehrlich carcinoma was also reported earlier by **Sahu *et al.* [31]**. They postulated that the loss of Mn-SOD activity could be due to the loss of mitochondria which leads to a decrease in total SOD activity in different tissues of the tumor host. It seems that oxidative damage caused by decreased capacity for H_2O_2 elemination is related to suppressed activity of CAT, as well as to suppressed direct antioxidant action of GSH. This is in agreement with the previous findings that CAT has a more significant role than GPx in protecting erythrocytes against oxidative stress. Some investigators have reported a higher $NO^{\cdot}$ synthase activity in tumors **,** while some have reported a lower activity**.** Our result supports the general observation that some malignancies are associated with an increased level of nitric oxide.

According to **Illmer *et al.* [32]**, fermented wheat-germ extract with standardized benzoquinone content has been shown to exert an intense antioxidant activity with no side effects**.** The reduction in free oxygen radicals induced by it is correlated with a clinically significant improvement in the quality of life in patients with advanced cancer **[33].**
FWGE also decreases nucleic acid ribose synthesis through the non-oxidative steps of the pentose cycle but increased a direct glucose oxidation through the oxidative steps thus limiting cell proliferation and protecting human cells from oxidative stress **[34].**

The results of our laboratory tests of kidney and liver function are in good agreements with **Sukkar *et al.* [33]** who reported that, the use of FWGE was safe and caused no alteration in renal and/or hepatic function.

Our findings of histopathological examinations are supported by the suggestion of **Hidvegi *et al*. [35]** who reported that FWGE has a marked inhibitory effect on metastasis formation in tumor-bearing animals**.** FWGE treatment resulted in a statistically significant decrease in the number of liver metastases of the 3LL-HH tumor inoculated into the spleen. In case of the HCR-25 human colon carcinoma, the 50 days of FWGE treatment decreased the amount of liver metastases, in addition to reducing the weight of the tumorous spleen. In case of the B16 melanoma inoculated into the muscle, also a significant decrease of 85% was observed in the number of metastases as compared to the control group.
Also, **Nichelatti & Hidvegi [9]** reported that, no patients treated with FWGE did show new metastases, neither hepatic, nor in other organs, while 4 patients (22%) did develop new metastases in the control group at the end of the study.
Moreover, **Comin-Anduix *et al.* [7]** demonstrated that, FWGE is a complex mixture of biologically active molecules with potent anti-metastatic activities in various human malignancies.

In the present study, measuring of apoptosis using Acridine Orange-Ethidium Bromide Staining of tumor of FWGE treated mice, **Fig** (6c-d), showed induction of apoptosis in the marginal region,with the presence of some of healthy tumor cells in the core region of tumor.
Telekes *et al.* [8] reported that, FWGE induces apoptosis in malignant hematologic and solid tumor cell lines and exhibits immunomodulatory activities.
Since apoptosis involves the killing of cancer cells, a major mechanism of FWGE action is apoptosis induction. FWGE influences apoptosis via several molecular pathways. FWGE induce apoptosis via poly (ADP-ribose) polymerase (PARP) and other pathways **[6].**

Our *in vitro* and *in vivo* results revealed that treatment with FWGE+ nano-Se mixture is more effective than FWGE. These results may be due to selenium nanoparticles and these results are in harmony with **Mueller *et al*. [36]** who stated that, FWGE appeared to be a good combination partner for drug regimens, in particular as modulator of drug activity and attenuator of drug toxicity. From the clinical and preclinical data, it is suggested that FWGE has single agent activity and appears to modulate (synergize) the effect of commonly used cytostatic and other anticancer drugs**.** Oral co-administration of FWGE inhibits tumor metastasis formation after chemotherapy and surgery in advanced colorectal cancers **[37].** Moreover, **Chen *et al.* [38]** reported that Nano-Se possesses great selectivity between cancer and normal cells and displays potential application in cancer chemoprevention and chemotherapy.
Many studies showed that Nano-Se exhibited novel in vitro and in vivo antioxidant activities through the activation of selenoenzymes **[39].**

Selenium is at various stages of clinical development as a chemopreventive agent based on published in vitro data demonstrating its ability to induce specific molecular perturbation associated with apoptosis and angiogenesis **[40].**
Our findings of high antioxidant effect of FWGE-nano-Se which caused marked increase in SOD, GPx, GSH and CAT and decrease inMDA and $NO_{(X)}$ are in accordance to **Shi *et al*. [41]** who reported that supplementation of selenium caused elevation in serum GSH-Px, SOD and CAT activities and decreased MDA in Se supplemented group (sodiumselenite, Se-yeast or nano-Se) than control ($P < 0.05$). GSH-Px, SOD and CAT activities notably increased in elemental nano-selenium compared with the other two Se supplementation groups.

Nano-Se exhibited an excellent bioavailability because of its high catalytic efficiency, strong adsorbing ability and low toxicity. All these specific properties of nano-Se and the different absorption pattern may explain the greater bioavailability of nano-Se compared with organic or inorganic Se **[42]**.

Selenium treatment can heal indomethacin-induced ulcers through prevention of lipid peroxidation and activation of radical scavenging enzymes, such as SOD, Cat, and GPx. So it was suggested that selenium is a powerful free radical quencher **[43].**

Also, the observed levels of these parameters were close to the normal values of the control. These results are in good accordance with those obtained by **El-Demerdash [44]** who found that Se maintained the levels of antioxidants, membrane-bound enzymes and the activities of antioxidant enzymes near normal levels, thus emphasizing their effects as antioxidants.

Moreover, **Rudenko [45]** reported that Se corrected the disturbance in liver antioxidative status of rats treated with aluminum trichloride.

FWGE-nano-Se improved liver and kidney function tests, these results are in accordance to **Biswas *et al*. [46]**, who studied the effect of oral administration of vitamin E and selenium on growth performance, haematological and biochemical parameters in Broiler Chicken at High Altitude and found that GOT and GPT values decreased significantly ($p<0.01$) in Se treated groups as compared to the control group without treatment. In agreement with **Soudani *et al*. [47]** who studied , the protective effects of selenium (Se) on chromium (VI) induced nephrotoxicity in adult rats and found that treatment with Se improve the creatinine and urea levels . The administration of Se in the diet of $K_2Cr_2O_7$ group protect the kidney function from chromium intoxication as indicated by a significant restoration of plasma urea, uric acid, creatinine as well as the creatinine clearance levels.

Furthermore, **El-Demerdash [48]** studied the antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium and stated that vitamin E or selenium alone proved to be beneficial in decreasing the levels of free radicals, lipids, urea, creatinine and increasing GST and the content of SH groups in plasma, liver, testes and kidney as compared with the negative control group. Also, they reported that Se alone had no significant effect on the activities of AST, ALT, as compared with the negative control group.

From our results treatment with FWGE-nano-Se decreased the rate of metastasis than treatment with FWGE alone. These results are in agreement with **Jakab *et al*. [37]** who reported that oral co-administration of FWGE inhibits tumor metastasis formation after chemotherapy and surgery in advanced colorectal cancers. The oral coadministration of FWGE with conventional treatments helped to improve the clinical outcome of colon cancer treatment when compared with treatment with conventional regimens alone and, at the same time, demonstrated no signs of toxicity. Also, **Hidvegi *et al*. [35]** studied the antimetastatic effect of FWGE alone or in combination with cytostatic drugs in a spleen-liver or muscle-lung mouse metastasis model using 3LL-HH, B16 and HCR-25 cell lines and found that in all three mouse models, the treatment with FWGE orally at 3 g/kg. b.w. daily dosage resulted in a significant reduction of liver or lung metastasis as compared to control mice. FWGE can be used as a supportive therapy in human cancer to reduce metastasis.

In the present study, measuring of apoptosis using Acridine Orange-Ethidium Bromide staining of tumor of FWGE-nano-Se mixture showed induction of apoptosis in both marginal and core regions in high ratio more than treatment with FWGE alone.

Combs and Gray [49] suggested that nutritional levels of selenium supplementation provide antioxidant protection against oxidative stress, while supranutritional levels may cause subtoxic effects to induce cell growth inhibition and/or apoptosis for cancer prevention **[50].** According to **Zeng *et al.* [51]**, Se treatment can alter several genes related to cell cycle/apoptosis in a manner related to cancer prevention. Treatment with Se resulted in the upregulation of genes involved in phase 2 detoxication enzymes, in certain Se-binding proteins and in some apoptotic genes.

ROS aregenerated as natural byproduct of normal cellular metabolism and has important roles in cell signaling. Intracellular ROS may attack cellular membrane lipids, proteins, and DNA and cause oxidative injury. Previous study had shown that free radicals could cause extensive chemical modifications and alterations in DNA and nucleoproteins, including modified bases and sugars and even strand breaks (genotoxicity) **[52].** Many chemopreventive and chemotherapeutic agents have been found to induce cancer cell apoptosis through upregulation of intracellular ROS generation **[53].** Growing evidences suggest that ROS generation acts as an important cellular event induced by Se compounds and resulted in cell apoptosis and/or cell cycle arrest **[54].** Treatments of Nano-Se generated a dose-dependent increase in intracellular ROS level, suggesting the involvement of ROS as a critical mediator in Nano-Se-induced cell apoptosis.

Several studies had also demonstrated selenite-induced apoptotic DNA laddering in the p53-mutant cancer cells without the cleavage of poly(ADP-ribose) polymerase (i.e., caspase-independent apoptosis); whereas metabolic precursors of CH_3SeH induced caspase-mediated apoptosis in those cells**.** However, selenite activated the caspase-mediated apoptosis involving both the caspase-8 and the caspase-9 pathways in the p53 wild-type cancer cells **[55].**

Conclusions

Finally, it could be concluded that our in vitro study indicated that FWGE-nano-Se has high cytotoxcity effect on EAC cells. In addition, our in vivo studies indicated that treatment of mice bearing tumor with FWGE-nano-Se induced tumor growth regression and showed antioxidant activity by increasing the deteriorated levels of GSH ,GPx , CAT and SOD in untreated groups and decreasing their elevated MDA and NO(X). Also, they have no side effect on liver and kidney function parameters.Moreover, it induced apoptosis in Ehrlich carcinoma cells and has antimetastatic effect.

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