Evaluation of galectin-3 gene Expression associated with Ehrlich tumor of female mice treated by Algerian Ephedra alata ethanolic extract

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Research Article

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Abstract

Background

Breast cancer is one of the most frequent cancers among females worldwide. *Ephedra alata* is a Saharan endemic that may be found in Algeria, North Africa, and other parts of the world. Recently it has become very used by Algerian patients to treat several diseases. Galectin-3 is highly expressed in a variety of neoplasms, and its expression has been linked to metastasis in various tumour cell systems.

Methods

The present study therefore was conducted to test the effect of *Ephedra alata* ethanolic extract (EEEA) on Swiss female mice induced with cancer. Determination of the functional consequence of galectin-3 expression in malignant mice breast cells is one of the main Target of the study. The expression of galectin-3 genes was examined in various groups of mice to investigate the involvement of galectins in breast cancer (negative control group, tumour without treatment as positive control, group treated with oral extract, group treated with injection of tumour with extract and protective group which take oral extract before induce of tumor).

Results

Size of tumor in induced mice with cancer cell were decreased and appear as capsulated after treatment with tested EEEA. The fold difference in Gal-3 relative to control was 32.8 in case of untreated tumor where it decreased in case of treatment with *Ephedra alata* extract and treatment using letrazol which used as reference drug.

Conclusion

The alcoholic extract of the *Ephedra alata* plant was shown to have a positive effect on the treatment of breast cancer cells in the investigation.

Background

Breast cancer is one of reasons which cause death in women. The incidence of breast cancer has gradually risen in latest years. Breast cancer has become one of the main illnesses that threaten the health of women **[1, 2]**. Although breast cancer treatment has produced progress, most patient fatalities are still metastasi. To develop more effective therapeutic strategies, a better understanding of the mechanisms of tumor metastasis is therefore important **[3–5]**. Over the previous 20 years, breast cancer diagnosis and therapy has been significantly enhanced and the development of fresh surgical therapy has been shown to be efficient **[5]**. Patients with breast cancer have a 5-year survival rate of about 50–

60%. After therapy, around half of all patients have recurrence and metastasis. Furthermore, late-stage breast cancer patients have an average survival period of 18 to 30 months. Despite the fact that several treatment strategies for breast cancer have been devised, virtually all of them have been proved to be ineffective. In addition, breast cancer tends to metastasize outside the breast, making it one of the hardest tumorto treat **[6]**. Surgery, chemotherapy, radiation treatment, and radiofrequency ablation have all made significant advancements in treating primary tumours. However, cancer progression to an invasive or metastatic stage is often associated with poor patient prognosis and remains a critical obstacle in disease treatment **[4]**. Although recent therapies have shown some potential for breast cancer, there is still no operational therapy for the mainstream of breast cancer patients in progressive stages. It is necessary to develop effective agents for slowing, reducing or reversing the incidence of breast cancer in women at high risk **[1, 2, 7, 8]**.

During cell division, traditional therapies can only kill tumor cells. But once the treatment is complete, tumor stem cell proliferation and differentiation will continue, leading to recurrence and metastasis **[9]**. Extensive research over the pastseveraldecades has identified numerous dietary and phytochemical compounds that have chemopreventive potential and couldrepresent an important source of anti-cancer lead molecules **[10]**.

There is a direct need for additional active and minus toxic therapeutic and protective approaches for breast cancers that can also neutralize the persistent occurrence of hormonal and targeted therapy conflict, which is the first-line therapy in the control of breast cancer patients.

Most review focuses on various bioactive chemopreventive and anti-cancer activities obtained from dietary sources (naturally present in fish) such as Omega-3 fattyacids **[10]**. Galectin-3, a member of the -galactoside-binding lectin family, is unknown despite its relevance in fibrosis, cancer metastasis, and development **[11, 12]**. Galectins are a low-molecular weight family of mannose-binding lectins with growth and cell activation functions for carcinoema. Anti-galectin antisera may inhibit rat cancer metastases and human melanomas **[13]**. Galectin-3 (Gal3) has a variety of roles in cancer genesis, progression, and drug resistance, depending on the characteristics of the tumour. Cancer stem cells (CSCs) are also connected to it **[14]**. The stochastic model posits that phenotypic variances between tumour cells are influenced by random internal and external causes, and that each cell has the same probability of transforming into a tumour **[15]**. Metastasizing tumour cells, as well as migratory-disseminating tumour cells and circulating tumour cells (CTCs) detected in the original tumour, were identified as prognostic indications. The signals of metastasis created on CTCs and migrant–spreading tumour cells have a better chance of distinguishing cell biology knowledge from systematic substructures of tumour cell dispersion and metastasis **[16]**.

Materials And Methods

Plant materials

The study is carried out using the aerial part (twigs and leaves) of Ephedra alata, that had been collected from Khenchela area in Algeria. The plant wasidentified and authenticated taxonomically by Miss Khanouf Hannane of the environment Department, Mohammed Esseddik Ben Yahya University, Jijel, Algeria.

Preparation of extract

The ethanolic extract was prepared by maceration of 50 g of plant powder in 150 ml of 70% ethanol. Then the two filtrates were recovered and mixed. Finally, the extract was evaporated at 50 °C using a Rotary evaporatortype (Laborota 400) and stored in refrigerator until used.

Experimental animals

A total number of 70 Swiss albino female mice, aged 8 to 10 weeksoldandweighting19.3–24.6 g, were used for solid Ehrlich tumor study. The rats were obtained from the Medical Research Institute (MRI) at Alexandria University (Egypt) and kept in polypropylene cages under temperature control (26°C) and a 12/12 h light/dark cycle. Throughout the trial, standard pellet feed and tap water were accessible at all times. Two weeks previous to the commencement of the testing, the animals were acclimatised to laboratory conditions. The request for assignment was agreed by the Committee on Institutional Animal Ethics. The animals were cared for according to the policies of the facility. The Alexandria Medical Research Center's Animal Care and Use Committee approved all animal handling and experimental methods. The Ehrlich ascites carcinoma cells were obtained from Alexandria University's medical research institution (MRI) (Egypt). Group I (C): negative control group, healthy mice, Group II (Er): tumor-bearing group, Group III (E): mice (non-tumor-bearing) were orally received Ephedra extract at dose of 1000mg/kg body weight (BW) per day for two weeks, Group IV(Er+L): mice were injected with Ehrlich ascites carcinoma cells. On the 10th day after injection, letrazol (reference anticancer drug) was orally administrated in saline water to mice at dose of 0.03125 mg/kg BW daily for two weeks, Group V (Er+Eo): mice were injected with Ehrlich ascites carcinoma cells. On the 10th day after injection, extract of Ephedrainsaline water (0.9%)was orally administrated to mice at dose of 1000 mg/kg BW daily for two weeks, Group VI (Er+Ei): mice were injected with Ehrlich ascites carcinoma cells, On the 16th day after injection, 0.5 ml of Ephedra extract was directly injected into solid Ehrlich tumor at dose of 1000 mg/kg BW day after day for 1 week and Group IIV (P):protective group, Mice were given Ephedra extract at a dosage of 1000 mg/kg BW twice a day for two weeks. The Ehrlich ascites carcinoma cells were then implanted into the right thoracic mammalian gland.

Evaluation of Tumor Growth

The tumour size was estimated during the experiment, and the tumour was removed from the tissue and weighed at the conclusion. The size of solid tumours was kept track of on a daily basis, and the tumour volumes and tumour growth inhibition rate were computed using the following formula (tccc): (length x width2)/2 Equals tumour volume (mm3), where length and width are measured in millimetres. Tumor

inhibition rate (%) = [(Average tumor volume of the control group - average tumor volume of the test group)/ average tumor volume of the control group] x 100.

Gene expression analysais

Gene expression analysais was performed in order to study changes in glactingene 3 in different treatment groups.

Quantitative analysis of galectins 3 gene expression using quantitative real time reverse transcriptase polymerase chain reaction (q RT-PCR)

Total RNA was isolated from solid tumors and normal mammalian glands using extraction kit (PureLinkTMARN Mini Kit invitrogen). qRT-PCR was implemented in a reaction mix of 20 µl using 10 µl 1-step QPCR SYBER mix (1X), 0.2 µl verso enzyme mix, 0-5.8 µl water (PCR grade) 1 µl forward and reverse primers (10 pm), 1 µl RT-enhancer, and 1-6.8 µl RNA template (50 ng). qRT-PCR platform was pragmatic as one cycle of cDNA mixture at 50°C for 15 min, one cycle foractivation of enzyme at 95°C for 15 min and followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 1 min and extension at 72°C for 30 sec. Primer used in the study{GAPDH housekeeping gene (GAPDH F: CCAAGAAGCTGAGCGAGTGTCTC ; GAPDH R : CCTGCT TCACCACCTTCT TG) & Glactin gene (Gal F : ACTTCAATCCTCGCT TCAA ; Gal R : GGCCACGCACTTAATCTT)}.

Statistical Analysis

Results as a means and standard deviation have been reported. Statistical analysis was performed using SPSS for all parameters of the study. Statistically significant values of $p<0.05$ were considered.

Results

Tumor growth

Measurement of both untreated and treated mammalian tumors were take place in the study to differantiate the changes in the different groups. The start of treatment was six days after the injection of the Erlish cells. Within the first 8 days of tumor development, control tumors (n = 10) increased in size by ~ 800 percent. Body mass of mouse were measured every 3 days, morphological phenomena were studied and tumour size were measurements (Table 1). Figure 1 Illustrate Experimental design of applications and animal groups (treated with Ephedra alata extract, treated with chemotherapy and untreated group) of the study.

Data are displayed as a mean ± SD. The difference in tumour size was significant and observed between groups II, IV, V, VI and IIV (tumour induced, no treatment) and (tumour induced, treated or pretreatment) subsequent to the regulation time (Fig. 2). Tumor volume (mm3) = (length x width2)/2, where the length and width are given in mm.

Tumor inhibition rate (%) = [(Average tumor volume of the control group – average tumor volume of the test group)/ average tumor volume of the control group] x 100.

Table 1
Size of tumor (mm) in the different studies group during the experiment groups of mice

group	0days	5 days	10 days	15 days	20 days	25 days	30 days
Group I (C)	-	-	-	-	-	-	-
Group II (Er)	-	-	0.0135 ± 0.004	0.258 ± 0.098	1.98 ± 0.78	3.12 ± 0.089 ± 0.94	4.678 ± 0.87
Group III (E)	-	-	-	-	-	-	-
Group IV(Er + L)	-	-	0.0141 ± 0,0022	0.253 ± 0.088	0.34 ± 0.097	0.42 ± 0.079	0.52 ± 0.098
Group V (Er + Eo)	-	-	0.016 ± 0.007	0.246 ± 0.095	0.249 ± 0.076	0.3 ± 0.098	0.354 ± 0.067
Group VI (Er + Ei)	-	-	0.0159 ± 0.007	0.276 ± 0.075	0.98 ± 0.087	1.22 ± 0.076	1.26 ± 0.11
Group IIV (P)	-	-	-	0.0136 ± 0.009	0.11 ± 0.01	0.13 ± 0.02	0.15 ± 0.1

Body masses of mice had no significant changes at the 1st 8 days compared to the control group. In a trail to identify the most promising changes between different groups under investigation comparing with control group, hematological and biochemical parameters were done (Table 2, 3). LDH and PLT were the most promising tests show the difference between tumor induced groups and control group where the two test were evaluated increase in tumor induce and decrease in different treatment groups. Studying the morphological appearance of the internal organs of all groups, it was found that all treated groups by the plant extract (*Ephedra alata*) whether they had tumor or not in existing health manifestations. Unlike chemicals treatments, they reduce immunity and make the shape of organs different from normal.

Table 2
Hematological parameters

	Treatment groups						
	Negative control	Positive control	Treated with extract without Erlish	Pretreated with extract	Treated with letrazol	Treated orally with extract	Treated by injection in tumor
RBCs	8.63 ± 0.52	4.62 ± 1.91	8.99 ± 0.39	8.86 ± 0.29	7.37 ± 0.91	7.34 ± 0.81	8.91 ± 0.61
HB	13.21 ± 0.92	7.71 ± 1.82	13.4 ± 0.39	12.83 ± 0.93	11.1 ± 0.56	11.46 ± 1.12	12.91 ± 0.52
WBCs	6.5 ± 0.83	13.1 ± 1.61	6.3 ± 0.75	11.92±	7.81 ± 0.75	14.1 ± 2.33	14.6 ± 0.98
PLT	482 ± 9.28	741 ± 21.39	493 ± 11.6	277 ± 29.35	357 ± 39.4	517 ± 21.32	425 ± 20.13

Table 3
Biochemical parameters

	Treatment groups						
	Negative control	Positive control	Treated with extract without Erlish	Pretreated with extract	Treated with letrazol	Treated orally with extract	Treated by injection in tumor
Total protein	6.8 ± 0.52	6.1 ± 1.31	7.1 ± 0.95	6.9 ± 0.37	6.1 ± 0.32	6.3 ± 0.31	5.4 ± 0.79
Urea	52.83 ± 8.21	55.32 ± 3.7	46.21 ± 1.09	49.92 ± 3.45	62.31 ± 2.19	53.21 ± 1.6	49.31 ± 1.21
Creatinine	0.2 ± 0.03	0.22 ± 0.01	0.21 ± 0.03	0.27 ± 0.04	0.28 ± 0.09	0.23 ± 0.06	0.34 ± 0.02
SGOT	287.38 ± 16.2	301 ± 21.3	430 ± 28.11	369 ± 17.3	691 ± 21.2	411.23 ± 29.2	501 ± 29.3
ALT	63.86 ± 5.8	84 ± 9.39	89 ± 2.39	86.11 ± 8.1	99 ± 12.6	89.2 ± 19.3	81 ± 17.3
LDH	3162 ± 29.89	4523 ± 39	3422 ± 21.9	4380 ± 34.9	19120 ± 47.9	5722 ± 21.9	9860 ± 31.9

Gene expression analysis

Expression related to untreated mamilian gland tissue in mice without induced tumors and treatment with ephedra extract were take place. The effect change in galactin3 fold of relative gene expression through the different treatment and comparison to control group. Two tissue of each treatment were used in the experiment of relative galactin 3 gene expression. After extraction each sample of RNA were used in

expression and repeat 2 time in the same run, after that calculation occurred using all reading and analysis as presented in Figures **(3, 4)**. For details of the statistical significance refer to the Results. Normal breast tissue expressed low levels (1) galectin-3. High expression (32.8) was found in untreated breast cancer. The expression fold of galectin-3 was significantly decreased in case of tratment groups [Group III (E), Group IV (Er + L), Group V (Er + Eo), Group VI (Er + Ei) and Group IIV (P)] to be (0.899, 0.154, 0.454, 0.711 and 0.544) respectively.

Discussion

Under standing the mechanism of action of dietary compounds or traditionally used herbal shaving potential preventive and therapeutic effects on cancer may provide a rationale for further translational studies **[17–19]**. The relevance of scientific validation of natural bioactive substances for clinical usage in the treatment repertoire for breast cancer is highlighted in a study by Daniela B et al [**10**]. Although the specific role of galectin-3 in cell-laminin interactions is unknown **[20–22]**, Vincent C et al **[11]** discovered that its expression was down-regulated in colon cancer tissues at both the protein and mRNA levels as illness progressed. Gene expression reporting has generated expression signatures from which predictive examinations may result in the creation of clinical conclusions in patients with breast cancer **[23–25]**. Some of these signatures are based on entire tumor tissue profiling (signatures of the tissue) **[16]**.

After isolation of mouse hematopoietic cells based on a variety of phenotypic markers based on Li Y et al **[26]** study, these results were later confirmed. According to Yuichiro Honjo et al **[27]**, galectin-3 expression is required to sustain the transformed and tumorigenic phenotype of MDA-MB-435 breast cancer cells. According to an analysis of galactin 3 gene expression, breast cancer cells have lower expression of galectin-3 in relation to their aggressiveness, which is consistent with previous findings **[28–30]**.

Conclusion

The study proved that the alcoholic extract of the *Ephedra alata* plant had a good effect on the treatment of cancer cells in the breast. It can also be given as a type of condom used for cancer, as taking simple and continuous doses prevented the formation of cancer cells despite injecting the breast with cancer cells, and this was evident in the shape of cells, biochemical parameter of blood and fold difference in Gal-3 genes by comparing all the groups under study.

Declarations

Ethics approval and consent to participate

The Pharmaceutical and Fermentation Industries Development Center in the City of Scientific Research and Technological Applications (SRTA-city) performed this study, which was authorized by its ethics committee (IACUCs) l IACU # 55-2x-02022.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Moufida BENSAM conceived the study and funded the project. **Safaa M. Ali** and **Moufida BENSAM** performed plant extraction, design of animal study, measurment, analyses of biological parameter, gene expression analysis and wrote the manuscript. **Hocine RECHRECHE** supervised and revised the manuscript and **Abeer E.Abdelwahab** Designed the experiment and revision the result. All authors were involved in reviewing and editing the final manuscript.

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Declaration of competing interest

The authors declare that they have no competing interests.

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Figures

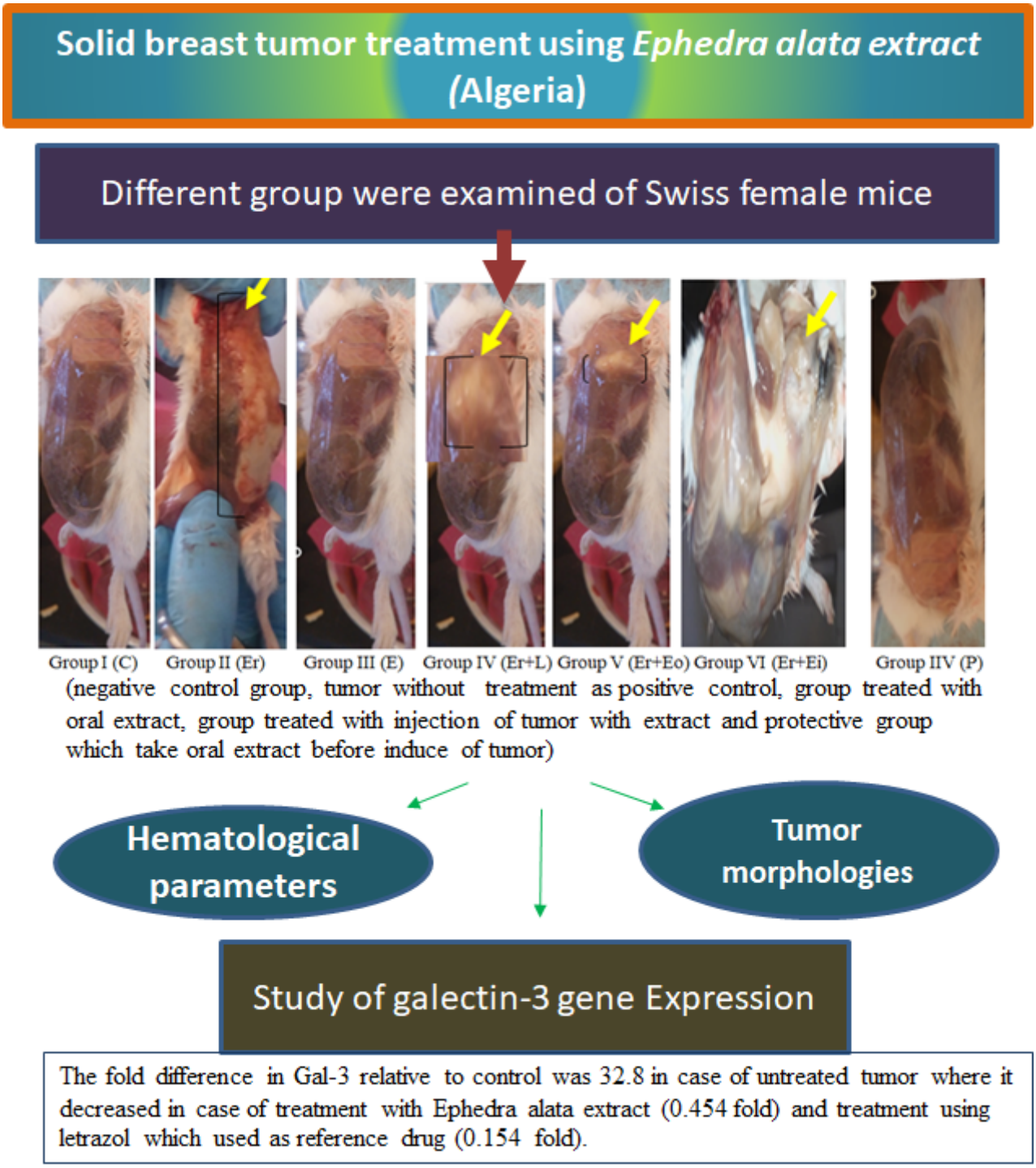

Figure 1

Experimental design of applications and animal groups (treated with Ephedra alata extract, treated with chemotherapy and untreated group) of the study

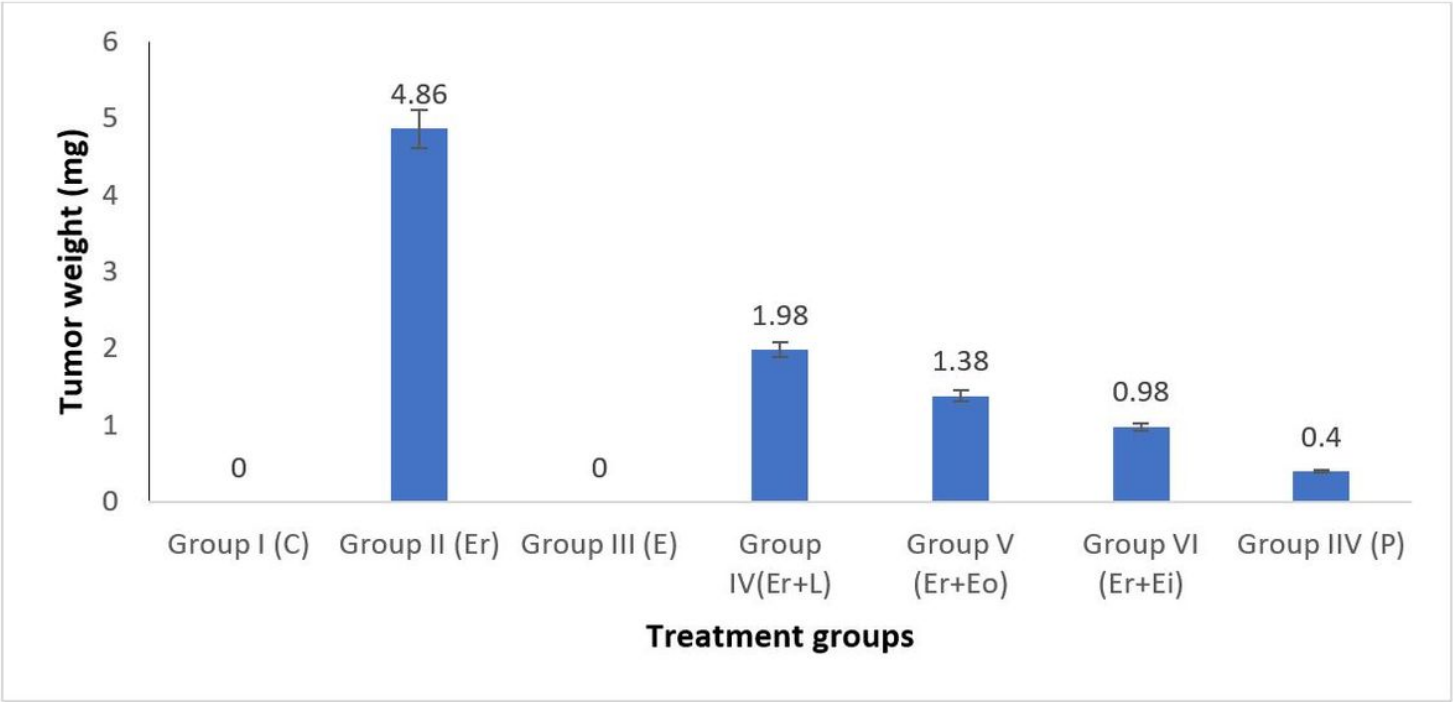

Figure 2

Incidence of tumor weight (mg) in the different group

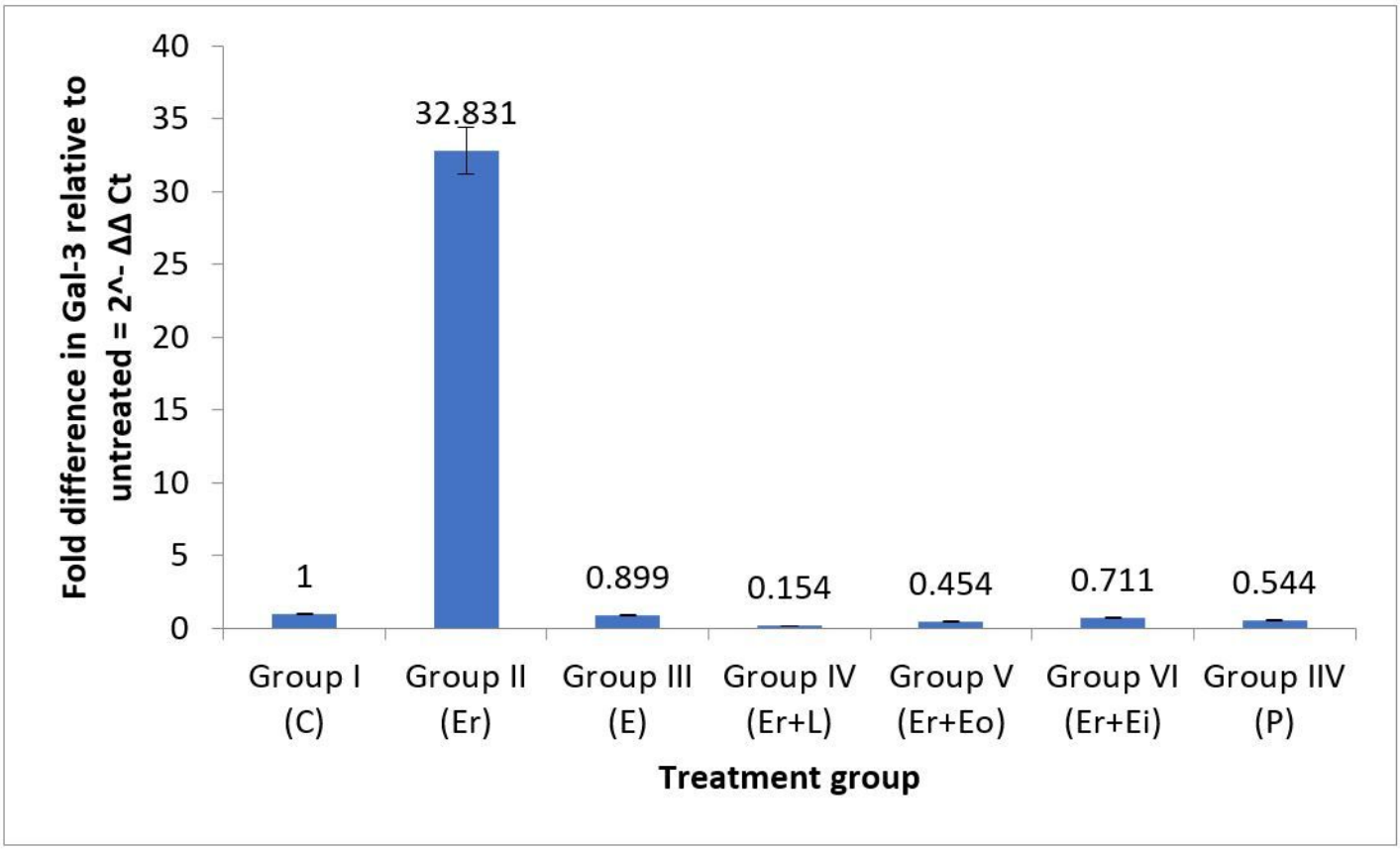

Figure 3

Gene expression level of galactin 3 during the experimental period

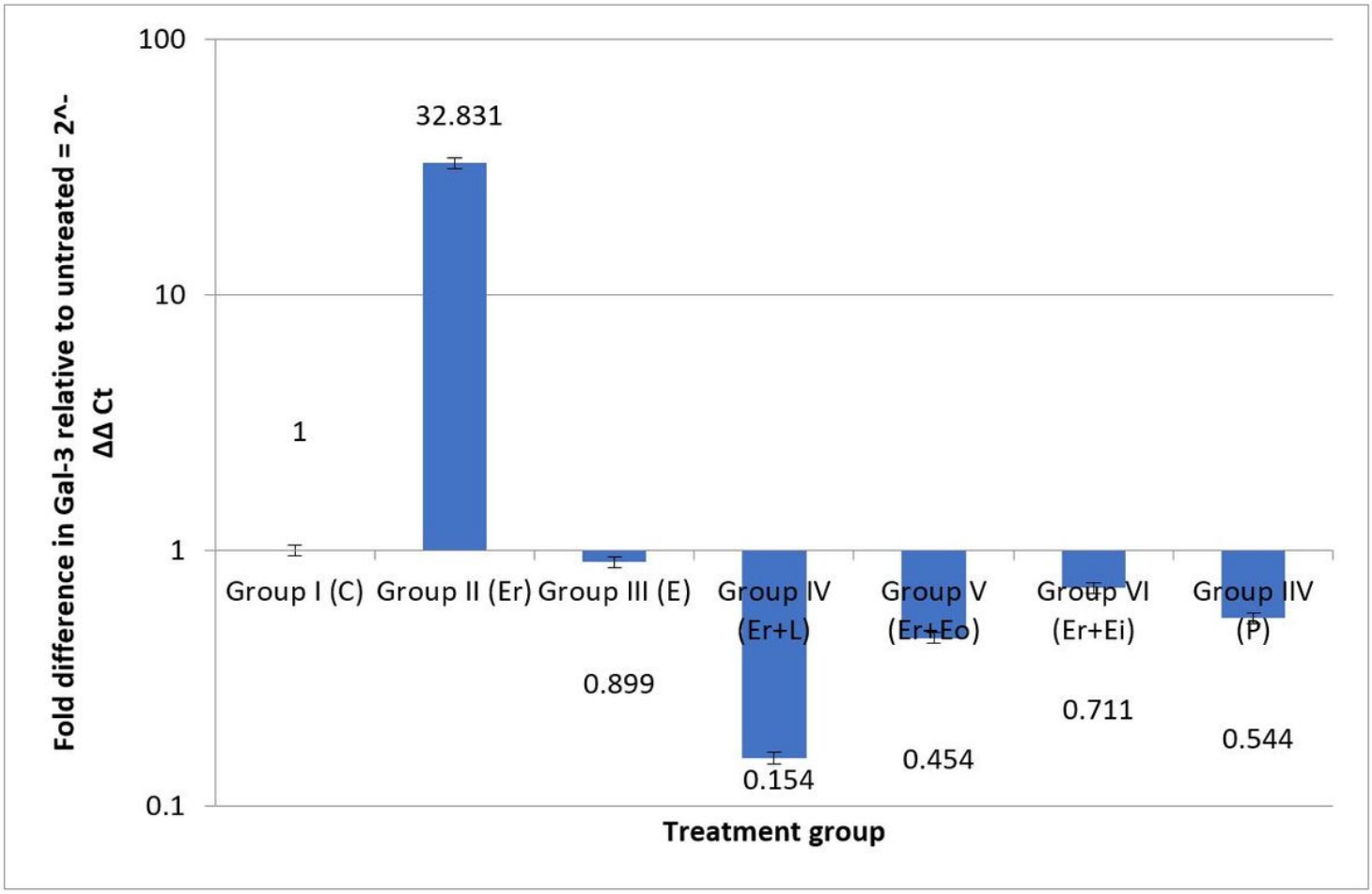

Figure 4

The effect change in log galactin3 fold of relative gene expression through the different treatment and comparison to control group