Abstract:

Aim: Numerous scientific studies have examined the relationship between safranal and its effect on various diseases including cancer. Yet, more studies needed to evaluate safranal consumption effect on epithelial- mesenchymal transition and induction of apoptosis in head and neck squamous cell carcinoma. The aim of current study is to investigate the effect of Safranal on the biological activities of laryngeal squamous cell SCC cel line; namely the epithelial-mesenchymal transition as well as viability of squamous cell carcinoma cells, therefore providing a more effective and less toxic possible treatment for this malignancy.

Methodology: Safranal was applied on laryngeal SCC cell line for 24, 48 and 72 hours respectively. cell viability was quantified using MTT assay. Apoptosis was measured by anti-apoptotic BCL-2 immunohistochemical marker. Finally, epithelial-mesenchymal transition was observed and evaluated using N-cadherin immunohistochemical marker.

Results: It was found that ultimate effects of safranal on laryngeal SCC cells at 72 hours, reduction cellular viability % in MTT assay and induction of apoptosis in cancer cells by inhibition of anti-apoptotic BCL-2. Additionally, it reduced the expression of N-cadherin marker which indicated inhibition of epithelia to mesenchymal transition.
**Conclusions:** Trial of consumption of safranal daily as a part of saffron and its application in cancer adjuvant treatment may contribute to the prevention and treatment of head and neck squamous cell carcinoma respectively.

**Keywords:** Safranal, Laryngeal Squamous Cell Carcinoma, Epithelial-Mesenchymal Transition, Apoptosis.

1. **Introduction:**

Cancer is considered as a crucial leading cause of mortality worldwide. Human head and neck squamous cell carcinoma (HNSCC) accounted for the sixth most common type of cancer worldwide, its rate of occurrence is exceeding half a million new cases annually. The associated 5-year survival rate was approximately 50%, so HNSCC had one of the lowest survival rate cancers. Although treatment was significantly improved over the past decades but the survival rate did not show great improvement. In the head and neck region, oral squamous cell carcinoma (OSCC) is one of the most frequent type of malignancies which originates from the mucosal epithelium of the oral cavity and oropharynx.

Up till now, the approach for treatment of OSCC is via conventional therapeutic methods which involve surgery, radiotherapy and chemotherapy. Unfortunately, conventional treatment modalities lead to therapy-related side effects both psychologically and somatically; thus, researchers made all their effort to search for natural alternative anticancer substances that have selective cytotoxic activity for the neoplastic cells and do not harm the normal cells so provide high efficacy and minimal side effects. Nearly most of innovative anticancer drugs originate from metabolites of plants or are phytochemical analogues.

Therefore, chemoprevention is supposed to be a fruitful adjuvant treatment of cancer for inhibition of both cancer growth and progression. Safranal is extracted from the dried stigmas of *Crocus sativus* L., which is valuable spice that has plentiful amount of carotenoids and it is also usually used in different parts of the world. It is mainly cultivated in Iran. Different biological and pharmacological activities of safranal have been studied by many researchers, as anticarcinogenic, antigenotoxic, antitumor, antioxidant, antidepressant and radical scavenging activities.

Apoptosis is a systematic and harmonized sequential process causing cell death. Balanced apoptosis is essential to reach homeostasis to avoid accumulation of genetic mutation or aged cells. Increased or decreased apoptosis leads to degenerative diseases or developing cancer respectively. Cancer cells resist this programmed cell death by its innate overexpression of inhibitor of apoptosis proteins. Bcl-2 family is a class of pro-apoptotic and anti-apoptotic proteins with Bcl-2 gene being recognized as one of the anti-apoptotic genes that inhibit apoptosis so facilitating accumulation of DNA errors leading to cancer formation.

Additionally, epithelial-mesenchymal transition (EMT) involves dynamic switch in cellular organization from epithelial to mesenchymal phenotypes, which causes functional alterations in cell migration leading to invasion. It is also vital biologic phenomenon during normal development as it manages the formation and development of various tissues as well as organs. Researchers discovered the relation between EMT and the pathogenesis of certain diseases, such as cancer, thus giving a big attention to identify effective therapeutic agents against it. EMT is characterized
by reduction of E-cadherin expression and abnormal expression of N-cadherin, which is resulting in elevating the invasiveness of human carcinoma cells and poor prognosis.

Furthermore, cadherin is a glycoprotein on the cell membrane which is necessary for intercellular adhesion and essential for embryo development and the maintenance of normal tissue. In relation to its distribution in tissues, cadherins have more than 10 subgroups. Cadherin-switch is a process that occurs in cancer, in which loss of E-cadherin in malignant tumors is accused of tumor growth and metastasis as intercellular adhesion is damaged due to mutation and genetic variation. Upregulation of N-cadherin is involved with epithelial-mesenchymal transition and correlated with invasion of malignant tumors.

Therefore, the current work was done to investigate the effect of safranal on cell viability, apoptosis and EMT on HNSCC human epithelial cell type 2 (Hep-2) cell line. As a result of few studies were done on safranal and HNSCC.

2. MATERIAL & METHODS

2.1. Material:

2.1.1. Cell Line

Laryngeal squamous cell carcinoma cell line, (HEP-2) was obtained from the International Center for Advanced Research (ICTAR-Egypt) supplied with compatible nutrient media. HEp-2 cells were obtained from the "American type Culture Collection" (ATCC) in the form of frozen vial with the reference number "CCI-23".

2.1.2. Reagents

Fetal Bovine Serum (FBS) was purchased from GIBO COBRAL® Limited, Scotland as a sterile serum in 500 ml bottle (stored at -20°C till used).

Phosphate buffer saline (PBS) was obtained from Sigma Aldrich, USA. Trypsin solution 0.25% in PBS, pH 7.5 was supplied from International Center for Advanced Research (ICTAR-Egypt).

Safranal: Safranal extract 90 % stabilized (W338907, SAMPLE-K, Sigma Aldrich-USA),

2.1.3. Antibodies:

BCL-2: CONFIRM mouse monoclonal primary antibody anti-bcl-2 (124) (Ventana, USA) IgG1 in PBS.

N-cadherin: Rabbit polyclonal anti-human N-cadherin/ CD325 antibody (E-AB-64011, Elabscience, USA), IgG in PBS with 0.02% sodium azid, 50% glycerol, pH 7.3 (Fig.7).
Positive control: Hep-2 cell line was used as a positive control for BCL-2 and N-cadherin according to manufacturer's instructions.

Negative control: Hep-2 cell line was prepared in the same method after omitting the step of the primary antibody application to ensure the specificity of the technique.

2.1.4. Universal Kit:

The ultraView Universal Diaminobenzidine (DAB) Detection Kit (indirect biotin free system/ horseradish peroxidase (HRP)-conjugated secondary antibody for detecting mouse IgG, mouse IgM and rabbit primary antibodies) was used.

2.2. Methods

In this study, 4 groups were used to evaluate the anti-cancer effects of safranal, one control group without any treatment and the other 3 groups differed in time of safranal application after 24-, 48- and 72 hrs.

2.2.1. Cytotoxicity Assay:

Methyl Thiazol Tetrazolium (MTT) assay is a quantitative colorimetric method to determine cell proliferation. It detects reduction and accumulation of yellow tetrazolium salt which is a water-soluble salt to an insoluble purple formazan complex within mitochondria of healthy cells only. In this study, HEp-2 cells were seeded in 96-well culture plates. Control wells were left untreated and treated wells were treated with a safe concentration of safranal 1.9 μM, then well plates were incubated for 24, 48 and 72 hours by dispensing of 50μL of 0.5mg/ml stock solution on the treated wells and incubated at 37°C for 4 hours. Thereafter, the treatment medium was gently removed from the wells, cells were washed using PBS and 50μL of DMSO were added to each well to dissolve the purple formazan crystals. The absorbance was conducted at 570nm using the Dynatech MR5000 spectrophotometer (Dynatech Laboratories, Inc., Chantilly, VA). The absorbance values at 570 nm were relative to the number of residual viable cells. Results were determined as the mean of three independent experiments 11. The viability percentage was calculated as follows:
Viability % = mean of tested cells/time mean of control %

2.2.2. Immunohistochemical Staining Procedures:

2.2.2.1. Cell Line Pellet Preparation:

Hep-2 cell line flasks were treated with safranal for 24, 48, and 72 hrs; one flask was left untreated as a control one. Then the detached and adhered cells of all flasks were harvested post treatment and using trypsination. Centrifugation of cell suspension was done by spanning at 1800 rpm for 10 minutes at room temperature. Then supernatant will be decanted. 5 ml of 70% ethanol was added for 30 minutes and vortexed mildly then the same steps were repeated using 100% ethanol until a very solid pellet was obtained. Finally, using a clean wooden applicator stick, cell pellets were pulled out carefully out of the tube and into a cassette lined with black biopsy filter paper.

These sections were stained with Hematoxylin-Eosin (H&E) then examined by light microscopy. The rest of cells was used for immunostaining, by using the same previous method; cells were mounted on positively charged (Opti-Plus) slides for immunostaining procedures with Bcl-2 and N-cadherin immunohistochemical markers. These slides provided better adhesion to the tissue sections and prevented them from sliding during staining procedures.

2.2.2.2. Steps of Basic Immunostaining Procedures:

Staining was automatically performed using Ventana Bench mark autostainer (USA) at the Pathology Department, National Cancer Institute; Cairo University, as follows:

- Rehydration of the tissue sections were done in descending grades of alcohol (100%, 90% then 70%) for 10 minutes then microwaving the tissue sections in 10 μM citrate buffer, pH 6.0 for 7-10 minutes followed by cooling at room temperature for 20 minutes (antigen retrieval step) then washed with the washing solution PBS. The sections were then incubated in 0.3% hydrogen peroxide for 30 minutes to block the endogenous peroxidase activity. The sections were washed by PBS before the application of 100 microns of Bcl-2 mouse monoclonal antibody at dilution of (1:100) under incubation temperature of 30 °C for 32 minutes. Other sections were washed by PBS before the application of 100 microns of N-cadherin rabbit polyclonal antibody at dilution of (1:100) under incubation temperature of 30 °C for 32 minutes.

- Then application of the secondary antibody for 30 minutes then washed by PBS. DAB was applied to sections for 15 minutes at room temperature then PBS. Counter stain with Mayer’s Hematoxylin was applied for 8 minutes and post counter stain with bluing reagent for 4 minutes. Slides were extracted and arranged in racks. Slides were washed in tap water for 5 minutes and then dehydrated in ascending grades of alcohol (70%, 90% then 100%) for 5 minutes in each container. Slides were cleared in xylene and then cover slips were applied and mounted using DPX mounting agent.

2.2.3. Assessment of Hematoxylin and Eosin Stained HEP-2 Cells and Immunostaining Interpretation for BCL-2 and N-cadherin:
Five microscopic fields of each slide were photomicrographed at the power of X400. This was done using a digital video camera, which was mounted on a light microscope. Then, images were transferred to the computer system for analysis. Field selection was based on the presence of the highest number of apoptotic cells and cells that regained intercellular junctions. The photomicrographs were evaluated for the presence of morphological criteria of apoptosis.

All the immunostained sections were examined by the image analyzer computer system using the software Leica Qwin 500 (Germany) at Center of Research and Dental Requirements, Faculty of Dentistry, Cairo University.

3. Results:
3.1. MTT Assay:

The cell viability percentage showed gradual decrease with increasing concentrations of safranal. The number of these cells in the control group was more than the treated cells with safranal. The lowest value of cell viability was found at the highest concentration of safranal (62.3μM), while the highest value of cell viability was found at the lowest concentration of safranal (0.31μM). IC 50 value was determined as 13.40 μM. The values were plotted as shown in figure 1 & table 1.

![MTT assay graph](image-url)

**Fig. 1:** A linear graph showing viability % of cells at different concentrations of safranal.

<table>
<thead>
<tr>
<th>Safranal Conc μM</th>
<th>Mean</th>
<th>Viability %</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.3</td>
<td>0.04</td>
<td>11.01</td>
</tr>
<tr>
<td>31.1</td>
<td>0.06</td>
<td>14.98</td>
</tr>
<tr>
<td>15.6</td>
<td>0.12</td>
<td>28.59</td>
</tr>
<tr>
<td>7.8</td>
<td>0.26</td>
<td>65.35</td>
</tr>
<tr>
<td>3.9</td>
<td>0.30</td>
<td>75.13</td>
</tr>
<tr>
<td>1.9</td>
<td>0.38</td>
<td>94.62</td>
</tr>
<tr>
<td>1</td>
<td>0.41</td>
<td>100.74</td>
</tr>
<tr>
<td>0.5</td>
<td>0.41</td>
<td>100.74</td>
</tr>
<tr>
<td>0.25</td>
<td>0.41</td>
<td>102.17</td>
</tr>
</tbody>
</table>
Table 1 showing variable concentrations of safanal with corresponding viability % of Hep-2 cells

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Viability %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.13</td>
<td>0.40</td>
</tr>
<tr>
<td>0.63</td>
<td>0.40</td>
</tr>
<tr>
<td>0.31</td>
<td>0.40</td>
</tr>
</tbody>
</table>

3.2. BCL-2

3.2.1. Morphological Assessment

It showed positive cytoplasmic and nuclear immunostaining in the control group (fig.2). In the 24 hrs and 48 hrs safranal groups some cells showed positive cytoplasmic immunostaining only, while others showed negative immunostaining (fig.3 and 4). While in the 72 hrs safranal group, the majority of cells were negatively immunostained with BCL-2 and only few cells showed faint positive immunostaining (fig.5).

Fig.2: A photomicrograph of the control group showing positive cytoplasmic and nuclear immunostaining of BCL-2 in epithelial-like cells (yellow arrow) (x400).
Fig. 3: A photomicrograph of 24 hrs safranal group showing positive cytoplasmic immunostaining of BCL-2 of some epithelial-like cells (yellow arrow) and other cells showing negative BCL-2 immunostaining (red arrow) (x400).

Fig. 4: A photomicrograph of 48 hrs safranal group showing positive BCL-2 cytoplasmic immunostaining of some epithelial-like cells (yellow arrow) with other cells showing negative immunostaining of BCL-2 (red arrow) (x400).
Fig. 5: A photomicrograph of 72 hrs safranal group showing negative BCL-2 immunostaining of most epithelial-like cells (red arrow) and few cells showed positive immunostaining (yellow arrow) (x400).

3.3.1. Statistical Analysis:

Statistical analysis was done using one-way ANOVA test to compare between BCL-2 immunostaining in all study groups: control group and safranal groups (24 hrs, 48 hrs and 72 hrs), which revealed a high statistical significance among all the study groups (*P value* <0.001). Bonferroni test one of the Post Hoc tests was used for multiple comparisons between safranal treated groups (24 hrs, 48 hrs and 72 hrs), which revealed high statistical significance between the 48 hrs and 72 hrs group values in comparison to the 24 hrs group. Similarly, a high statistical significance between the 72 hrs group in comparison to 48 hrs group was also obtained (fig. 6).

Fig. 6: Column chart showing mean area percent of BCL-2 immunostaining in different study groups.
3.4. N-cadherin

3.4.1. Morphological Assessment

The stained sections were examined under the microscope to evaluate immunostaining of anti-EMT marker N-cadherin. In the control group, it showed positive cytoplasmic and nuclear immunostaining (fig. 7). In safranal groups: the 24 hrs, 48 hrs and 72 hrs groups some cells showed positive immunostaining both cytoplasmic and nuclear, while most of cells showed only positive cytoplasmic immunostaining (fig. 8, 9 and 10).

Fig. 7: A photomicrograph of the control group showing positive cytoplasmic and nuclear immunostaining of N-cadherin of epithelial-like cells (yellow arrow) (x400).
Fig. 8: A photomicrograph of 24 hrs safranal group showing positive N-cadherin cytoplasmic immunostaining of epithelial-like cells (red arrow) and few cells showing both cytoplasmic and nuclear immunostaining of N-cadherin (yellow arrow) (x400).

Fig. 9: A photomicrograph of 48 hrs safranal group showing positive N-cadherin cytoplasmic and nuclear immunostaining of epithelial-like cells (yellow arrow). Some cells show only positive N-cadherin cytoplasmic immunostaining without nuclear (red arrow) (x400).

Fig. 10: A photomicrograph of 72 hrs safranal group showing positive cytoplasmic immunostaining of N-cadherin of epithelial-like cells (red arrow). Some cells show both positive N-cadherin cytoplasmic and nuclear immunostaining (yellow arrow) (x400).

3.4.2. Statistical Analysis:
Statistical analysis was done using one-way ANOVA test to compare between immunostaining of N-cadherin in all study groups, it revealed no statistical significance between the 24 hrs safranal group and the control group. Comparison between 48 hrs safranal group and control group was statistically significant ($P$ value < 0.05), while the 72 hrs safranal group was highly statistically significant in comparison to the control group. Bonferroni test was used for multiple comparisons between safranal treated groups (24 hrs, 48 hrs and 72 hrs), which revealed no statistical significance between 24 hrs and 48 hrs groups ($P$ value > 0.05). Meanwhile, the 72hrs group showed high statistical significance in comparison with the 24 hrs group ($P$ value < 0.001). Additionally, the 72 hrs group was statistically significant in comparison with the 48 hrs group with $P$ value < 0.05 (fig. 11).

![N cadherin](image)

**Fig.11:** Column chart showing mean area percent of N-cadherin immunostaining in the different study groups.

4. **DISCUSSION**

Lip and oral cavity cancers are reported to be the 15th most common cancers worldwide according to the Global Burden of Disease. Up till now, screening strategy was not proved to be 100% accurate, while careful physical examination remains the primary approach for early detection.

Thanks to chemopreventive agents that are made from natural products that are capable of inducing apoptosis, inhibiting cell proliferation or modulating signal transduction. Besides cancer treatment plans, combination of multiple chemopreventive agents with multiple targets is now considered to be more effective than a single agent and considered a fundamental treatment of cancer side by side with surgery, radio and chemotherapy.

Saffron is a famous spicy that consumed in different parts of the world it is derived from the dried red-dark stigmas of *Crocus sativus* L. Several studies have shown various biological and pharmacological activities of saffron and its bioactive constituents (as safranal). *Crocus sativus* has been used to treat several medical conditions, such as gastrointestinal disorders, urological infections, as well as in
treating malignancies, also it has been shown to induce apoptosis in human cancer cell lines. Thus, safranal seemed like a promising chemotherapeutic agent in cancer treatment. However, the exact molecular mechanism of safranal action was not clear 5,16.

MTT viability assay was used due to its high sensitivity and reliability in evaluating the cell viability. MTT draws a linear relationship between metabolically active cells and the color produced by them, thus giving an accurate quantification of changes in the rate of cell death or proliferation, so it was used to screen the action of drugs and anticancer agents 17.

Safranal induced apoptosis and inhibited cell growth as well as proliferation with increasing concentrations. Safranal caused >50% cytotoxicity on Hep-2 cell line at a concentration of ≥ 15.6 μM, while < 50% cytotoxic activity was detected at concentrations 7.8 μM and 3.9 μM (table 1). Close results on OSCC cell line were also found by this study 5.

In addition, nearly a 100 % cell viability was observed at 1.9 μM making it the safest concentration of safranal in this study with an IC50 value of 13.40 μM coinciding with many studies that proved these effects of safranal on various cancer cells as prostate SCC 18, OSCC 5, colorectal carcinoma and hepatocellular carcinoma 19.

In the current study, immunohistochemistry was used to investigate the expression of two different markers, namely BCL-2 as an antiapoptotic marker and N-cadherin as an EMT marker in Hep-2 cell line. BCL-2 was the first discovered gene shown to promote prolonged cell survival rather than increased proliferation. Overexpression of BCL-2 is a common finding in leukemias, lymphomas and many cancer types 20,21,22. N-cadherin is a calcium-dependent adhesion protein that has been demonstrated in many human tumors with de novo expression, re-expression, up-regulation and down-regulation. N-cadherin is mainly overexpressed in leiomyoma, pheochromocytoma, adrenocortical carcinoma and mesotheliomas as well as in the most invasive and de-differentiated breast cancer cell lines 23. However, the role of BCL-2 and N-cadherin in SCC Hep-2 cell line and effect of safranal on the expression of both markers has not been investigated yet. For the best of our knowledge, this is the first study to evaluate the expression of BCL-2 and N-cadherin and their role in the biological behavior of Hep-2 cell line whether treated with safranal or not.

In this study; the difference in BCL-2 expression was highly statistically significant (p<0.001) between all study groups. Where BCL-2 expression was the highest and significantly increased in untreated control group of Hep-2 cell line. This was previously supported by study which found that Bcl-2 interacted with cellular molecules that inhibited apoptosis in laryngeal carcinoma 24. Additionally, it was concluded that overexpression of BCL-2 in patients with OSCC leads to dysregulation of apoptotic mechanism, thus making BCL-2 accused of oral carcinogenesis 25.

Additionally, Safranal treated groups (24 hrs, 48 hrs and 72 hrs) revealed a high statistical significance among all the study groups (P value <0.001) and within groups. Some cells of the 24 hrs and 48 hrs safranal groups showed positive cytoplasmic immunostaining only, while others showed negative immunostaining. Regarding the 72 hrs safranal group, the majority of cells were negatively
immunostained with BCL-2 and only a few cells showed faint positive immunostaining. This was supported by a study which concluded that safranal-induced apoptosis was clearly observed by the increased Bax (pro-apoptotic protein) to Bcl-2 ratio in hepatocellular carcinoma cells. This ratio was increased after safranal application in a time-dependent manner from 6 hrs till 48 hrs with the highest ratio at 48 hrs. Another study also showed the same results on gastric adenocarcinoma cell line after increasing the time of safranal application, the expression of BCL-2 was decreased.

Regarding N-cadherin, the control group showed positive cytoplasmic and nuclear immunostaining in all sections. However, the exact molecular mechanism that was responsible for N-cadherin translocation into the nucleus was largely unknown. There were few studies in the literature that reported nuclear N-cadherin expression in oral cancer cells. Researchers demonstrated that N-cadherin staining was mainly cytoplasmic in the neoplastic cells with some focal nuclear positivity. They also mentioned that nuclear pattern of N-Cadherin expression was particularly observed in dedifferentiated OSCC and unfortunately characterized by a worse prognosis.

Besides, another study delineated that nuclear N-cadherin up-regulation was required for mammary epithelial cell migration during EMT induced by transforming growth factor beta (TGF-b1). Additionally, researchers found that nuclear expression of N-cadherin was strongly associated with adverse prognostic factors, including T classification, N classification (TNM staging system), clinical staging, in addition to the overall survival. Moreover, they observed that the higher nuclear N-cadherin expression in the late stage than the early stage as well as associated with poorer survival rate in patients with the late stage of nasopharyngeal carcinoma. Therefore, the pattern of cadherin expression may be a useful diagnostic and prognostic tool in the evaluation of tumors and for defining the histogenesis of tumor cells.

Interestingly, same study noted that altered localization of N-cadherin expression has a differential effect on the prognosis of nasopharyngeal carcinoma patients. Regarding cytoplasmic expression of N-cadherin, there was no difference in the survival rate between patients with high and low expression. In contrast to nuclear expression of N-cadherin, inverse correlation between nuclear N-cadherin expression and favorable prognosis was noted as follows: patients with high nuclear N-cadherin staining had shorter overall survival than those with low N-cadherin expression.

Researchers stated that N-Cadherin expression is upregulated in the most invasive and de-differentiated breast cancer cell lines and this exogenous expression in tumor cells causes induction of increased cellular motility, invasion and metastasis. N-Cadherin also enhances the systemic dissemination of tumor cells by enabling circulating tumor cells to be accompanied with the stroma and the endothelium at distant sites though has poorer prognosis. Whereas the 5-year survival rate of patients with N-cadherin-positive tumors was significantly lower than that of patients with N-cadherin-negative tumors. Other researchers proved that elevated cytoplasmic or nuclear N-cadherin expression at the invading margin in several samples, especially in areas of tumor budding of nasopharyngeal carcinoma.

Regarding safranal groups: the 24 hrs, 48 hrs and 72 hrs groups some cells showed positive immunostaining both cytoplasmic and nuclear, while most of cells showed
only positive cytoplasmic immunostaining. Most studies have proved that N-cadherin was mainly localized in the membrane and cytoplasm of cancer cells of: breast cancer 29, laryngeal SCC 30 and prostate carcinoma 31. N-cadherin protein can also be localized in the nucleus of human umbilical vein endothelial cells during the endothelial-to-mesenchymal transition 32.

The results revealed that no statistical significance was found between the 24 hrs safranal group and the control group (P value >0.05). This may be accounted for the short time (24 hrs) of safranal application which had minor effects on EMT. While comparison between 48 hrs safranal group and control group was statistically significant (P value < 0.05), as well as the 72 hrs safranal group which was highly statistically significant in comparison to the control group, so this means that increasing time of safranal treatment decrease EMT of cancer cells. This was also confirmed by multiple comparisons between safranal treated groups, which revealed no statistical significance between 24 hrs and 48 hrs groups (P value > 0.05). The 72hrs group showed high statistical significance in comparison with the 24 hrs group (P value <0.001) and statistically significant in comparison with the 48 hrs group with (P value < 0.05).

5. Conclusion:

Safranal may be an effective chemopreventive agent in prevention and treatment of HNSCC by exerting apoptotic effect on cancer cells and inhibiting EMT. On other hand, more studies on different concentrations of safranal or usage of safranal nanoparticles, are needed to be done to evaluate these effects in vivo and clinical trials.

6. References:


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