**Journal of Home Economics**

***Volume 29, Number (1), 2019***

***http://homeEcon.menofia.edu.eg***

**Journal of Home Economics**

**ISSN 1110-2578**

# Study the effect of turnip leaves on tumor cells in vitro and in vivo

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**Abstract:**Vegetables and its by-products become and still play an important role in human nutrition and health, they are rich in phytonutriceuticals as vitamins, minerals, dietary fiber, phytochemicals and antioxidants. So, this work was carried out to study the effect of feeding tumoredmice on 5% turnip (*Brassica rapavar.rapa L*) leaves powder on tumor cell volume, cell viability, oxidative stress, antioxidants enzymes activities and functions of liver and kidney in female Swiss Albino mice. The results showed that the turnip leaves powder had high nutritional value; which it is rich in minerals, fiber, phenolic and flavonoid compounds. Moreover, as a result of feeding tumored female mice on turnip leaves powder 5% caused a significant*($p \leq 0.05$)* decrease in volume and count (cell viability) of tumor cell for both preventive and therapeutic groups compared to the positive control group. Meanwhile, the therapeutic group revealedthe highest percentage of inhibitory for tumor cell. Moreover, the results indicated that the levels of MDA were significantly decreased, and levels of SOD and CAT were significantly*($p \leq 0.05$)* increased for both preventive and therapeutic groups compared to the positive control group in liver tissues homogenate. Furthermore, liver enzymes and kidney function as urea nitrogen and creatinine of tumored female mice were decreased as a result of feeding on turnip leaves powder. So, the therapeutic group feeding on 5% turnip leaves had the lowest levels of all these analyses compared to the positive control group. Finally, turnip leaves powder and its ethanolic extract had antitumor activity against MCF-7 cells (human breast cancer cell line)*in vitro* and Ehrlich ascites carcinoma (EAC) *in vivo*. Also, it enhances the antioxidants enzymes activities in addition, turnip leaves powder caused improvement in liver and kidney function in female Swiss albino mice. So, it can be usedin preparing traditional foods for prevention and supplementation foods for cancer patient during therapeutic period.

**Key words:** Turnip leaves, MCF-7, Ehrlich ascites carcinoma, liver function, MDA.

**Introduction**

Turnip (*Brassica rapavar.rapa L.*) is considered one of the oldest cultivated vegetables that has been used for human nutrition, folk medicines and food technology (**Liang *et al.*, 2006 and Thiruvengadam*et al.* 2014)**.its belonging to the family of Brassicaceae which are widely consumed all over the world and have more attention because it have phytochemical components which play good role in human nutrition (**Akhlaghiand Bandy 2010)**. Roots, leaves, stems and flower buds are considered edible parts **(Fernandes *et al.*, 2007).**Turnip is rich in its contentof glucosinolates and phenolic compounds. Glucosinolates (GSLs) contain three major chemical classes as indolicglucosinolates (IGSLs), aromatic and aliphatic (AGSLs), where it consist of amino acids astryptophan, phenylalaninenand methionine, respectively. In general, there are over 100 types of glucosinolates, nearlythirty of them were found in Brassicas.(**Matthew *et al.*, 2015 and Kastell*et al.*, 2015).** In Egypt, turnip roots only is usually used for nutritionbut nowadays leaves also become used in making traditional dishes. Many studies revealed that high consumption of cruciferous vegetables significantly reduces the risk of certain types cancer and cardiovascular diseases, because it have phytochemical components which appeareantioxidant, antimicrobial and anticancer activity (**Thiruvengadam and Chung, 2015)**. **Ibrahim *et al.* (2014)**stated that the rats of cancer incidence in Egypt in 2013 per hundred thousand person were 166.6 for both males and females which liver, breast and bladder wereoccupied the first three positionsby 23.8, 15.4and6.9%, respectively for males and females. Moreover, for females breast cancerwill occupy the first positions32.0% By 2050. Which, the percentage of cancer incident will increase 3-fold in comparison to 2013. Although, chemotherapeutic drugs considered an important treatment for different types of cancer, but many synthetic drugs causes harm side effects as nephrotoxicity, neurotoxicity, infertility, thromboembolic complications, hair loss, nausea, and myocardial infarction. Epidemiological studies revealed that turnip have the protective effect against cancers, antihypertensive, antidiabetic, antioxidant, antiinflammatory, hepatoprotective, and nephroprotective this may be refer to its content of bioactive compounds as glucosinolates and isothiocyanates, flavonoids, phenolics, indoles and volatiles for

chemotherapeutic purposesbecause of their safety andefficacy efficiency (**Rybak *et al.*, 2009; Gupta *et al.*, 2013; Shabaruddin*et al.*, 2014 and CA *et al.*, 2019).**Ehrlich ascites carcinoma (EAC) is characterized by a quickly growing carcinoma with very aggressive behavior **(Gupta *et al.*, 2004).** So, EAC is a transplantable neoplasia from a malign epithelium, which coincides to mammary adenocarcinoma in female mice. When injected intraperitoneally (I.P) it grows in a form of an ascitic and when injected subcutaneously it grows in a solid tumor form**(Freitas *et al.*, 2006).**Tumor cell death can occur by many mechanisms such as cell cycle arrest, necrosis, apoptosis and autophagy (**Taatjes*et al.*, 2007).**

So, the aim of this research was studyingthe chemical composition and identified phenolic compounds in turnip leaves. Also, studying the cytotoxicity effect of turnip leave powder and its ethanolic extracton breast cancer tumor cells*in vitro* and *in vivo*.

**Materialsandmethods**

**Materials**

Fresh turnip (*Brassica rapavar.rapa L.*) leaves were collected from local agricultural area in Sharkia Governorate, Egypt, during December 2018.

Fourty adult female Swiss albino mice weighting (25±3g) were purchased from breeding unit of the National Research Centre, Dokki, Cairo, Egypt. Mice were housed in steel mesh cages under the normal laboratory condition and were fed on standard diet for one week before starting the experiments as an acclimatization period. Food and water were provided ad labtium and checked daily.

Casein, corn oil, starch, choline chloride, vitamins, minerals, and some kits for biochemical analysis were obtained from El-Gomhoryia, company Cairo, Egypt. Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Tumor cell lines: MCF-7 cells ( human breast cancer cell line) was obtained from VACSERA Tissue Culture Unit. Cairo. Egypt. Also, Ehrlich ascites carcinoma (EAC) cells were obtained from the National Cancer Institute, Cairo, Egypt

**Methods**

**Preparation of turnip leaves powder**

The fresh turnip leaves were washed and dried by sun drying. And it was grounded by an electric blender (Moulinex, LM207041 Super Blender, France) and was packed in low-density polyethylene bags until it was used for the required analysis.

**Preparation of turnip leaves ethanolic extract**

Dried powder of turnip leaves was extracted by aqueous ethanol 70% at a ratio (1:10 w/v) overnight at room temperature with shaking followed by filtration through filter paper, then the filtrate was evaporated in a rotary evaporator (BÜCHI-water bath-B-480, Germany) at 45°C. Extracts were freeze- dried at -60°C (Thermo- Electron Corporation - Heto power dry LL300 Freeze Dryer, France). The freeze dried crude extract was stored at (-5 °C) until further use. As described by **Contini *et al.* (2008**)

**Chemical analysis**

Turnip leaves powder was analyzed chemically for moisture, protein, fiber, ash, and oil (Fresh weight) contents were determined according to the methods described by **AOAC (2005)**. Some phenolic and flavonoid compounds were identified by using HPLC as described by**Goupy*et al.*, (1999)**.

**Evaluation the anti-tumor activity ( The *in vitro* study)**

**Cell line Propagation**

The cell line were propagated in Dulbecco's modified Eagle's medium (DMEM) fortified with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. Cells were kept at 37ºC with 5% $CO_2$in a humidified atmosphere incubator. Also, it was sub cultured two times a week.

**Theviability test determination**

Determination of cytotoxicity assay; the cells were implanted in 96-well plate by a cell concentration of $1\times10^4$ cells per well in 100µl of growth medium. Crude ethanolic extract of dried turnip leaves was added to fresh medium by different concentrations after 24 h. Serial 2-fold dilutions of the tested ethanolic extract were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) by micro pipette. The plates were stored at 37ºC with 5% $CO_2$ for 24 h in a humidified incubator. Each concentration of the

test sample were done in triplicate. Meanwhile,control cells were incubated without test sample. After incubation of the cells at 37°C, for 24h, the viable cells were determined by a colorimetric method according to**(Mosmann, 1983 and Gomha*et al.*, 2015)**.

**Biological experimental study (The *in vivo* study)**

**Experimental design**

Fourty adult female Swiss albino mice weighting (25±3g) were kept under normal healthy conditions and fed on basal diet which prepared according to (**Reeves,*et al.*, 1993**) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin mixture component was that recommended by **(Campbell, 1963)** while the salt mixture used was formulated according to **(Hegsted, 1941)** for one week. Then, mice were divided randomly into 4 groups (n = 10) as:

**Group (1): Negative control group**

This group was fed on basal diet along the experimental period.

**Group (2): Positive control group**

This group was injected intraperitoneal ( I.P) with Ehrlich ascites carcinoma (EAC), (2.5 x $l0^6$ cells/ 0.3 ml/mouse) and fed on basal diet along the experimental period according to **(Mazumdar, *et al.*, 1997 and Amer, 1986).**

**Group (3): Preventive group**

At first, this group was fed on turnip leaves powder by 5% of basal diet for 15 days then mice were injected I.P. by EAC cells (2.5 x $l0^6$ cells/ 0.3 ml/mouse) and feeding for anthers 15 days to be 30 days.

**Group (4): Therapeutic group**

At first, this group was injected I.P. with Ehrlich ascites carcinoma cells (EAC) by (2.5 x $l0^6$ cells/ 0.3 ml/mouse). Then, mice were fed on basal diet containing 5% turnip leaves powder for 15 days from the next day of implanting tumor cells to be 30 days.

**Sampling**

At the end of the experiment 30 days, mice were fasted overnight 12 h, then sacrificed under ether anesthesia. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature.Then, the blood was centrifuged at 4000 g for 10 minutes,the serum sampleswere carefully separated into dry clean

ependorf tubes by using a Pasteur pipette and kept frozen till analysis at -20°C.

EAC cells were harvested from each mouse in centrifuge tube containing heparinized saline to determine the volume and count of EAC cells (viability tests) according to the method described by **Melima*n et al.* (1997).**

Liver tissues were excised from each mouse and part of them were homogenate with saline for analysis.

**Biological analysis**

**Viability test:**viability test was carried out according to method of**Melima*n et al.* (1997)** using trypan blue exclusion method.

Antioxidant status in liver homogenate:superoxide dismutase (SOD) and Catalase(CAT) were assayed according to the method of **Sun *et al.* (1988)** and **Aebi(1984)**, respectively. While,Malondialdehyde (MDA) was determined according to the method of **Satoh (1978).**

Determination of liver function in serum: Alanine Amino transferase (ALT) and aspartate amino transferase(AST) were assayed by the methods of **Srivastava *et al.* (2002)** and **Chawla (2003).** Determination of total protein was carried out according to the colorimetric method of **Henry (1974).**

Determination of kidney function in serum:urea nitrogen was determined in serum according to the method described by **Patton and Crouch (1977).** creatinine concentration was determined as described by **Henry (1974).**

**Statistical Analysis**

The results are recorded as mean ± SD and subjected to analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system.Comparison among means were performed using the LSD test. The differences were considered significant at the 5% level ( $p \leq 0.05$) by using **(Costat version 6.311,1998-2005).**

**Results and discussion**

Data in Table (1) showed the chemical composition of dried turnip leaves powder (TLP), the moisture content of turnip leaves powder was 10.71%,total protein was31.86%.Fat and carbohydrate content were, 2.88% and 24.04%, respectively. Moreover, ash and crude fiber content were14.96 % and 15.55%, respectively. It is observed from the data that turnip leaves powder had high content of ash, protein and fiber which

suggest these use as functional ingredients in food technology. These results are in line with those reported by **Nasef (2018)**found that chicory leaves powder had high content of ash and fibers. **Abd El-Rahman *et al.* (2018)** displayed that the Molokiha leaves powder had high contents of fiber, ash and protein. Also, Moringa oleifera Lam. ( *M. oleifera* ) has high content in proteins, ash and essential amino acids **(Kou *et al.*, 2018). Also, Povolo*et al.*, (2019)** cleared that *Brassica rapa* are rich in amino acids.

**Table (1): Chemical composition of turnip leaves powder ( g /100g dry weight basis)**

| Item | Quantity (g/100g) |
|---|---|
| Moisture | 10.71±0.25 |
| Protein | 31.86±1.41 |
| Fat | 2.88±0.08 |
| Carbohydrate | 24.04±0.91 |
| Ash | 14.96 ± 0.13 |
| Crude fiber | 15.55±0.53 |

Turnip leaves had significant amounts of phenolic (bioactive) compounds, which provide many health benefits beyond basic nutrition. This refer to the antioxidant activity (**Silva *et al.*,2004**). Data in Table (2) evident that the phenolic compounds in turnip leaves powder (*Brassica rapa L*). It is clear to notice that the highest phenolics compounds of dried turnip leaves recorded for gallic acid, rutin , querectin, naringenin and chlorogenic acid which the values were 346.13, 101.62, 92.86, 89.87 and 78.98mg/100g DW, respectively. All of these bioactive play an important role for improving the human health by participating in the antioxidant defense system against free radical generation. These results are in line with **Romani *et al.*(2006) and Fernandes *et al.* (2007**) found that turnip leaf contains isorhamnetin, kaempferol and quercetin glycosides. Moreover, **Shafek*et al.*(2018)** detected by chromatographic a new natural isorhamnetin glycoside called isorhamnetin 3-O-β-D-glucopyranosyl(1‴′→2‴)-α-L-hamnopyranosyl(1‴→2″)-βglucopyranoside (L2) with 15 flavonoid compounds known as kaempferol glycosides, quercetin glycosides,

isorhamnetin glycosides and aglycones. And **Povolo*et al.*** **(2019)** identified by HPLC several bioactive polyphenols and flavonoids compounds as quercetin and rutin, succinic acid and alanine was found in leaves of *Brassica. rapa* plants.

**Table (2): Identified phenolic compounds in turnip leaves powder by HPLC**

| Identified compounds | Conc. ( mg / 100g DW) |
|---|---|
| Gallic acid | 346.13 |
| Chlorogenic acid | 78.98 |
| Catechin | NI |
| Coffeic acid | NI |
| Rutin | 101.62 |
| Ellagic acid | ND |
| Coumaric acid | 23.85 |
| Vanillin | NI |
| Ferulic acid | 45.12 |
| Naringenin | 89.87 |
| Propyl Gallate | 8.51 |
| Querectin | 92.86 |
| Cinnamic acid | 2.46 |
| Total | 789.4 |

NI: Non identified .

The anti- tumor effect of turnip leaves ethanolic extract against MCF-7 cells (human breast cancer cell line) *in vitro* study are shown in **Table (3) and Fig 1**, which the results cleared that the percentage of inhibitory of breast cancer cell line increased whereas, the percentage of viability cell decreased by increasing the concentration of turnip leaves ethanolic extract. Moreover, the $IC_{50}$ is the concentration required to make toxic effects in 50% of intact cells. Which, the inhibitory activity against breast carcinoma cells was detected under these experimental conditions to be 207 ± 4.6 µg/ml. The obtained results are in line with **Aipire*et al.*** **(2018)** studied the anti-tumor activity of *Brassica rapa L* against A549 cells (lung carcinoma cell lines) with different doses 200, 400 and 600 µg/mL of BR for 24 h, the cell number decreased compared with untreated cells. Also, BR reduced the viability of A549 cells in a dose- and time-dependent manner significantly. The IC50 values of BRBS at 24h was 429.4 µg/ ml. **Shafek*et al.*(2018)** Studied the

antitumor effect of aqueous extract of turnip leaves and its nano silver against M -NFS-60 cells (human Mouse Myelogenous Leukemia carcinoma) and HELA cells (human Cervical cancer cell line) and found that nano silver turnip leaf extract had high inhibitory for both two cell line. **Chung *et al.* (2016)** found thathairy root cultures of turnip can inhibit growth of both colon and breast cancer cell lines and this may be refer to its rich content of antioxidants andglucosinolates (GSLs) compounds.

**Table (3):Evaluation the cytotoxic effect of turnip leaves extractagainst MCF-7 cell line**

| Sample conc. (µg/ml) | MCF-7 cell line ( breast cancer cell line) | | |
|---|---|---|---|
| | Viability % | Inhibitory % | S.D. (±) |
| 0.00 | 100.00 | 0.00 | 0.00 |
| 3.90 | 100.00 | 0.00 | 0.00 |
| 7.80 | 100.00 | 0.00 | 0.00 |
| 15.60 | 100.00 | 0.00 | 0.00 |
| 31.25 | 97.18 | 2.82 | 0.34 |
| 62.05 | 89.06 | 10.94 | 0.57 |
| 125.00 | 68.72 | 31.28 | 2.46 |
| 250.00 | 40.35 | 59.65 | 2.71 |
| 500.00 | 19.47 | 80.53 | 1.95 |
| **$IC_{50}$** | **207 ± 4.6 µg/ml** | | |

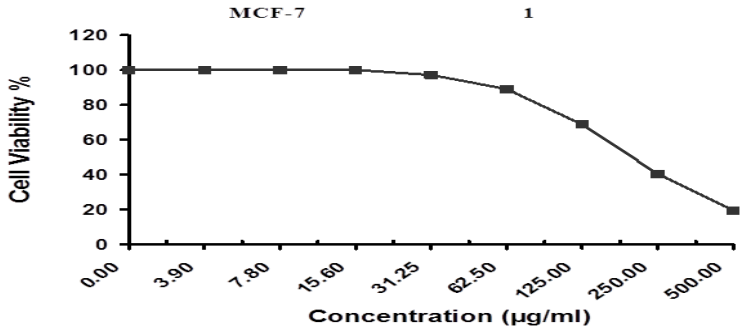


**Fig.(1): Evaluation the cytotoxic effect of turnip leaves extract against MCF-7 cell line**

The tumor volume and count for mice studied groups were illustrated in **Table (4).** The mean volume of EAC in the positive control group was found to be 6.67 ± 0.76 (ml). Treatment with turnip leaves powder (5% of basil diet) caused a significant decrease in tumor volume reached to 3.5 ± 1.32 and 2.50 ± 0.5 (ml) by 47.53% and 62.52% at ($P \leq 0.05$) for both preventive and therapeutic groups, respectively compared to the positive control group.Concerning tumor count (viability assay), it is observed that treatment tumored mice groups by feeding on turnip leaves powder showed significant decrease ($P \leq 0.05$) in tumor count to be 76.97±7.86 and 49.9±6.30 ($x10^6$) by 40.78% and 61.61% for both preventive and therapeutic groups, respectively; compared to the positive control group 129.97± 9.57($x10^6$). It is cleared from the obtained results that feeding tumored mice on turnip leaves powder caused a significant decrease in both volume and count which this may be refer to its rich content in phenolics, flavonoids compounds and high antioxidants activity. So, when applied to EAC cells it had a beneficial effect which reduced the volume and the viability of EAC cells because most of these compounds had anti- tumor activity.Many studies revealed that consumption of cruciferous vegetables suppress tumor progression compared to other vegetables and fruits intake **(Keck and Finley,2004).** Broccoli, turnip, rutabaga, cabbage, kale, brussels sprouts and cauliflower consider the main dietary sources of cruciferous vegetables **(USDA, 2002)**, Which cruciferous vegetables have anticancer effects because it have bioactive compounds includingsulforaphane, isothiocyanates, glucosinolates, phenolics compounds and flavonoids copounds (**Hecht,2002 and Ambrosone*etal*.,2004)** which it can inhibits the proliferation of malignant tumor cells by some mechanisms as cell cycle arrest or apoptosis or /and autophagy (**Singh *etal*., 2005 and Herman-Antosiewicz, *etal*.,2006 and Arumugam and AbdullRazis, 2018**). Programmed cell death(Apoptosis) it is one of the mechanisms which conserved cellular suicidal program to facilitate the removal of the damaged and dysfunctional cells**(Sangkari*et al*., 2012)**. Moreover, apoptosis play an important role in cancer treatment which it occurs by three different stages: condensation of nuclear heterochromatin, changes in cell membrane by shrinkage and changes in the orientation of intra cytoplasmic organelles **(Chamond*et al*., 1999).AbdullRazis and Mohd**

**Noor (2013)** found that glucosinolates could induce apoptosis to suppress of cancer progress. Also, **Liang *et al*.,(2008)** revealed that treatment human lung adenocarcinoma (LTEP-A2) cells by sulforaphane caused significant decrease in cell growth and G2/M-phase arrest . **Herman-Antosiewicz*et al*.(2006)** stated that sulforaphane-induced autophagy in human prostate cancer cells (PC-3 and LNCaP). The obtained results are in line with **Nabila (2012)** cleared that, balanitoside isolated from *Balanitesaegyptiaca* have lowered a significantly the number of EAC cells by 62.76% and 61.17% and increase in life span of EAC bearing mice by 36.72% and 13.63% for preventive and therapeutic groups, respectively.

**Table (4): Effect of turnip leaves powder on tumor volume and count inmice studied groups.**

| Groups | Tumor volume | | EAC cells count | |
|---|---|---|---|---|
| | Volume(ml) | % of Change | Count ($x10^6$) | % of Change |
| Negative control group | ـــ | ـــ | ـــ | ـــ |
| Positive control group | $6.67^{a}$ ± 0.76 | 0.00 | $129.97^{a}$ ± 9.57 | 0.00 |
| Preventive group | $3.50^{b}$ ± 1.32 | -47.53 | $76.97^{b}$ ± 7.86 | - 40.78 |
| Therapeutic group | $2.50^{b}$ ± 0.5 | -62.52 | $49.9^{c}$ ±6.30 | - 61.61 |
| LSD | 1.85 | | 16.03 | |

Values are denoted arithmetic means ±standard deviation of the mean. Means with different letters in the same column differ significantly at ($P \leq 0.05$).

Data illustrated in **Table (5)** show that transplantation EAC cells into normal mice caused a significant increase in lipid peroxidation malondialdehyde (MDA) level to be149.33±9.50 nmol/mg compared to the negative control group (healthy mice) 34.03±3.65 nmol/mg. Moreover, treatment tumored mice with turnip leaves powder 5% of basil diet caused a significant decrease in the MDA levels for both preventive and therapeutic groups to be 67.67±9.29 and 30.33±7.77 nmol/mg by 54.68% and 79.69% respectively compared to the positive control group. The effect of treated tumored groups by turnip leaves powder on SOD enzyme levels in liver homogenate are recorded in **Table (5)**. The mean value of the positive control groupwas12.17±2.75 U/mgwhich lower than the negative control group 42.27±0.75 U/mg.

which the results showed significant difference with percent of increasing by 247.33% of the positive control group. All treated groups showed a significant increase in (SOD) enzyme levels by 76.66% and 152.01% for both preventive and therapeutic groups respectively, as compared to the positive control. Also, the mean value of CAT enzyme was found to be 78.0±2.00 ng/mg for the negative control group. These values were decreased significantly in the positive control group to be 26.67±1.26 ng/mg. Meanwhile, treatment tumored mice by turnip leaves powder results in a significant increase in CAT enzyme levels were being 40.76±2.24 and 54.33±4.16 ng/mg by 52.83% and 103.71% for both preventive and therapeutic groups, respectively; compared to the positive control group. It is cleared that transplantation of EAC cells into normal mice caused a significant increase in MDA and decrease in both SOD and CAT which a marker to enhanced lipid peroxidation by free radicals which lead to oxidative stress for cells. While, treatment by feeding mice on turnip leaves powder which rich in natural antioxidants caused a significant enhance in antioxidants defense system. The obtained results are matched with **Mori*et al.*(2018)** demonstrated that cruciferous vegetables as turnip which rich in isothiocyanates can reduce oxidative stress which activation of procarcinogens and enhance the excretion of carcinogens. Also, there is a positive correlation between cruciferous vegetable intake and decrease the percentage of cancer mortality for both men & women. **Nasef (2018)** revealed that treatmenthepatotoxicity rats by different levels of chicory (*CichoriumIntybus L.*) leaves powder caused a significant increase in antioxidants liver enzymes as SOD and CAT.

**Table (5): Effect of turnip leaves powder on antioxidants status in liver tissue homogenate for mice studied groups**

| Groups | MDA (nmol/mg) | % of Change | SOD (U/mg) | % of Change | CAT (ng/mg) | % of Change |
|---|---|---|---|---|---|---|
| Negative control group | $34.03^{c}$± 3.65 | — | $42.27^{a}$±0.75 | — | $78.0^{a}$± 2.00 | — |
| Positive control group | $149.33^{a}$± 9.50 | 0.00 | $12.17^{d}$± 2.75 | 0.00 | $26.67^{d}$± 1.26 | 0.00 |
| Preventive group | $67.67^{b}$± 9.29 | -54.68 | $21.50^{c}$± 1.80 | 76.66 | $40.76^{c}$± 2.52 | 52.83 |
| Therapeutic group | $30.33^{c}$± 7.77 | -79.69 | $30.67^{b}$± 3.06 | 152.01 | $54.33^{b}$± 4.16 | 103.71 |
| LSD | 14.89 | | 4.29 | | 5.09 | |

Values are denoted arithmetic means ±standard deviation of the mean. Means with different letters in the same column differ significantly at (P≤0.05).

Results presented in **Table (6)** cleared that transplantation of EAC cells into normal mice caused a significant decrease (P≤ 0.05) in serum total protein levels compared to the negative control group. On the other hand, feeding tumored mice on turnip leaves powder 5% of basal diet caused a significant gradual increase in total protein by 12.69% and 13.45 % for both preventive and therapeutic groups, respectively compared to the positive control group. While, there was non-significant differences between preventive and therapeutic groups in AST. Concerning ALT enzyme activity, it is observed that the treatment tumored mice with turnip leaves powder caused a significant decrease in ALT enzyme levels for both preventive and therapeutic groups by 33.33% and 50.34% compared to the positive group whereas, there was non-significant differences in ALT enzyme levels between therapeutic group and the negative control group. Also, transplantation of EAC cells into normal mice caused a significant increase (P ≤ 0.05) in serum AST enzyme activity level compared to the negative control group, treatment tumored mice by turnip leaves powder induced a slight significant improvement in levels of AST enzyme activitycompared to the positive control group and there was non-significant differences between both preventive and therapeutic groups. The obtained results are in line with **Hassanpour-Fard*etal.* (2018)** found that treatment diabetic rats with aqueous extract of turnip leaves at doses 200 and 400mg / kg b.w caused a significant improvement in liver enzymes compared to the positive control group.

**Table (6): Effect of turnip leaves powder on serum liver function for mice studied groups.**

| Groups | Total protein (g/dl) | % of Change | ALT (U/L) | % of Change | AST (U/L) | % of Change |
|---|---|---|---|---|---|---|
| Negative controlgroup | 6.39$^{a}$±0.05 | ___ | 25.13$^{c}$±1.36 | ____ | 156.60$^{c}$±5.76 | ___ |
| Positive control group | 5.28$^{c}$±0.10 | 0.00 | 51.70$^{a}$± 5.82 | 0.00 | 247.20$^{a}$±2.86 | 0.00 |
| Preventive group | 5.95$^{b}$±0.12 | 12.69 | 34.47$^{b}$± 3.81 | -33.33 | 232.80$^{b}$±1.9 | -5.83 |
| Therapeutic group | 5.99$^{b}$±0.10 | 13.45 | 25.67$^{c}$± 3.20 | -50.35 | 230.47$^{b}$±3.35 | -6.77 |
| LSD | **0.21** | | **7.32** | | **7.06** | |

Values are denoted arithmetic means ±standard deviation of the mean. Means with different letters in the same column differ significantly at (P≤0.05).

Data illustrated in **Table (7)** show the effect of feeding tumored mice on basal diet containing turnip leaves powder on kidney function, there was a significant differences (P≤0.05) in serum urea and creatinine levels between the negative control group and the positive control group. While, treatment tumored mice by feeding on 5 % of TLP caused a significant (P≤0.05) decrease in serum urea and creatinine levels being (12.67±1.53 and 12.0±1.0 mg/dl) and (0.41 ± 0.01 and 0.34 ± 0.02 mg/dl) by ( 23.68% and 23.42% )and (43.84% and 53.42%) respectively, compared to tumored group(+ve). While, non-significant differences found between (preventive group and therapeutic group) and (therapeutic group and negative group), respectively for urea and creatinine levels in serum. So, it is observed from the data that turnip leaves powder caused a significant improvement in kidney function for tumored mice compared to untreated group.

**Table (7): Effect of turnip leaves powder on serum kidney function for mice studied groups**

| Groups | Kidney function | | | |
|---|---|---|---|---|
| | **Urea mg/dl** | **% of Change** | **Creatinine mg/dl** | **% of Change** |
| Negative control group | $10.33^{c}$± 0.58 | ___ | $0.37^{c}$± 0.04 | ____ |
| Positive control group | $15.67^{a}$± 0.85 | 0.00 | $0.73^{a}$± 0.12 | 0.00 |
| Preventive group | $12.67^{b}$± 1.53 | - 23.68 | $0.41^{b}$± 0.01 | - 43.84 |
| Therapeutic group | $12.0^{bc}$± 1.0 | - 23.42 | $0.34^{bc}$± 0.02 | - 53.42 |
| LSD | **1.88** | | **0.13** | |

Values are denoted arithmetic means ±standard deviation of the mean. Means with different letters in the same column differ significantly at (P≤0.05)

## Conclusion:

In conclusion, the present study has demonstrated that turnip leaves powder had high nutritional value as protein, minerals, crude fiber and bioactive compounds which play an important roleto enhance the antioxidants defense system and reduce oxidative stress. Turnip leaves powder and its extract show cytotoxicity effect against tumor cell line *in vivo* and *in vitro*.Furthermore, more research must be done on the future on turnip leaves for applications in human diets, food industrial and medical industrial instead of the synthetic antioxidants/chemicals used which have induced health hazards and side effects for the human being.

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*Journal of Home Economics, Volume 29, Number (1), 2019*

# دراسة تأثير أوراق اللفت على الخلايا السرطانية بيولوجيا ومعمليا خارج الجسم

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**الملخص:**

تلعب الخضروات ومخلفاتها دورًا هاما في تغذية وصحة الإنسان، فهي غنية بالمغذيات النباتية مثل الفيتامينات،المعادن،الألياف الغذائية،الكيماويات النباتية ومضادات الأكسدة. حيث تم إجراء هذا البحث لدراسة تأثير تغذية إناث الفئران السويسرية المصابة باالسرطان على مسحوق أوراق اللفت بمعدل 5 ٪ من الوجبة الأساسية على حجم و عدد الخلايا السرطانية ، الجهد التأكسدي، نشاط الإنزيمات المضادة للأكسدة وكذلك وظائف كل من الكبد والكلى. أوضحت النتائج أن مسحوق أوراق اللفت له قيمة غذائية عالية حيث يحتوي على نسبة عالية من المعادن، الألياف،المركبات الفينولية والفلافونيدات. علاوة على ذلك، أدت تغذية إناث الفئران المصابة بالخلايا السرطانية على مسحوق أوراق اللفت بمعدل 5٪ الى حدوث انخفاض معنوى في حجم وعدد الخلايا السرطانية لكل من المجموعات الوقائية والعلاجية مقارنة بالمجموعة الضابطة الموجبة، فى حين اظهرت المجموعة العلاجية تاثيرا عاليا لتثبيط نمو الخلايا السرطانية. علاوة على ذلك ، أشارت النتائج إلى أن مستويات MDA حدث لها انخفاضا معنويا فى حين حدوث زيادة معنوية فى نشاط الإنزيمات المضادة للأكسدة لكل من SOD و CAT لدى المجموعات الوقائية والعلاجية مقارنة بالمجموعة الضابطة الموجبة فى خلايا الكبد. بالإضافة الى حدوث انخفاضا معنويا فى نشاط إنزيمات الكبد وكذلك وظائف الكلى فى السيرم متمثلة فى مستويات اليوريا والكرياتينين بالنسبة للفئران المصابة بالسرطان نتيجة التغذية على مسحوق أوراق اللفت. أخيرًا، أظهر مسحوق أوراق اللفت نشاطًا مثبطا على معدل نمو الخلايا السرطانية(الأرليش) لدى إناث الفئران السويسرية وكذلك من خلال الدراسة المعملية أظهر المستخلص الإيثانولى لمسحوق أوراق اللفت نشاطا مثبطا لخلايا سرطان الثدى(MCF-7)، بالإضافة الى تعزيز نشاط الإنزيمات المضادة للأكسدة وكذلك حدوث تحسن في وظائف الكبد والكلى. لذلك يوصى باستخدامه فى اعداد الأطعمة التقليدية، الأغذية التكميلية لمرضى السرطان خاصة سرطان الثدى خلال الفترة العلاجية.

**الكلمات المفتاحية:** أوراق اللفت ،وظائف الكبد ، خلايا الأرليش السرطانية ،المانوالدهيد.