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# Correlation between Polyphenolic Contents of Fennel Seeds and its Mixtures with Green Tea on Cardiovascular Disease and Cytotocity Activity

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**Abstract:**
Goal of the Review In this review, we examine the significance of nitric oxide (NO) and summaries the research on the relationship between cardiovascular disease (CVD) and cognitive impairment. The (NO) route is a mode of causation. recent discoveries Epidemiological studies have found evidence linking midlife CVD and related risk factors to an increased risk of dementia and cognitive decline later in life. The cause of this association is unknown, but risk may be communicated by an increase in neuro degeneration (such as amyloid deposition), vascular changes (such as small vessel disease), and mechanistically due to increased levels of oxidative stress and inflammation as well as changes in NO bioavailability. Among the groups of the rats fed on fennel and green tea 9:1 (G4; 16.21 ± 0.54 µM) slightly reduced the NO level in serum, compared with that, group fed on fennel and green tea 4:1 (G5; 14.14 ± 0.81 µM). Obtained results showed fennel with green tea as ratio 4:1 have a significant activity as antitumor agents against the tested human hepatocellular liver carcinoma cell lines without effect on normal cells. This study characterizes antioxidant activity and phenolic compounds of traditional medicinal plants associated with antioxidant, the improved ABTS•+ DPPH method was used to systematically assess the total antioxidant capacity.

**Keywords:** Fennel**;** green tea; cardiovascular disease; nitric oxide; antioxidant

## Introduction

During the last decades, the beneficial health effects of green tea have been demonstrated in a number of studies. Tea drinking is associated with reduction of serum cholesterol, prevention of low density lipoprotein (LDL) oxidation and a decreased risk of cardiovascular disease and cancer (**Mackay ,Blumberg 2000, Chung *et al* 2003** ). Due to these facts the popularity of this beverage has grown all over the world. The

chemistry of tea is complex: polyphenols, alkaloids, amino acids, glucides, proteins, volatile compounds, minerals and trace elements (**Stagg, Milin 1975**). Polyphenols are the most biologically active group of tea components which have antioxidative, antimutagenic and anticarcinogenic effects (**Higdon, Frei 2003; Yao *et al* 2004**). But there are other compounds in green tea with interest for human health like fluoride, caffeine, minerals and trace (**Cabrera *et al* 2003; Powell et *al* 19986; Hope *et al* 2006**) elements like, chromium and manganese. The regular consumption of tea can contribute to the daily dietary requirements of some of these elements (**Xie et *al* 1998)**. Due to the importance of minerals in tea several studies have been carried out in order to determine their levels in tea leaves and infusions (**Fung *et al* 1999 ; Matsuura *et al* 2001 ; Fernandez –Caceres *et al* 2001 ; Behrendt *et al* 2002**).

Fennel (*Foeniculum vulgare Mill.*) is a small genus of annual, biennial or perennial herbs distributed in central Europe and Mediterranean region. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as a culinary spice (**Díaz-Maroto et *al* 2006; Rather *et al* 2012**). Mature fennel fruit and its essential oil are used as flavoring agents in food products such as liqueurs, bread, pickles, pastries, and cheese. They are also used as a constituent in cosmetic and pharmaceutical products (**Rather *et al*., 2012; Telci *et al* 2009**).

Seeds are commonly used for homemade remedies, being useful in the treatment of several complaints, specifically those of the digestive system. Fennel is also highly recommended for diabetes, bronchitis and chronic coughs, for the treatment of kidney stones, and is considered to have diuretic, stomachic and galactogogue properties (**Camejo-Rodrigues *et al* 2003; Carvalho, 2005; Novais *et al* 2004; Salgueiro, 2004**).

## Material and methods

### *Chemicals*

### *Plant collection*

The plants were obtained at a local market in in Zagazig Government, Sharkia, Egypt, and the botany department of the Faculty of Science at Zagazig University identified it. The voucher specimen for the green tea was (20180905) and the voucher specimen for the fennel was (2Fvu2) sample was deposited for further references.

### *Plant extraction*

Plants were dried for seven days at room temperature. Then it finely ground into a fine powder. Green tea and fennel aqueous extract was prepared by the cold maceration method. Green tea powder and fennel

powder was soaked in alcohol, kept in a flask at room temperature for two weeks. Ten the extracts was filtered and stored at room temperature for further investigations. Apart of Green tea and fennel extract was put on the Rotary apparatus (evaporation) to vapor to produce a solid gum that was then tested by phytochemical and chemical composition analysis.

***Phytochemical Screening***

Preliminary phytochemical screening of the extract was carried out to identify the active constituents, using standard methods. Phytochemical screening for flavonoids, alkaloids and coumarins of both seeds of fennel and leaves of green tea **( Selim & Abd El-Azim, 2020**).

***Chemical composition***

The chemical composition at temperature 220 °C, the humidity rate is 23% and further dried in an air the result is shown in table 1&2. Grinded fine powder of dried leaves of green tea and dried fennel seeds , dried Ethanol extract and were used for analysis of total composition; total protein % , total fat % , Total fiber % , humidity % and total ash % .

***Biological Evaluation***

***Antioxidant activity assay***

**DPPH Test**

In vitro antioxidant assays used in this present work for the antiradical pharmacological evaluation of the green tea and fennel are the following ABTS test (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolonization assay) and DPPH test (2, 2-diphenyl-1-picrylhydrazyl free radical bleaching assay) which are two of the most common and famous assays used as a base for the further biological evaluation of the antioxidant activities of new organic compounds. Antioxidant activity assay by DPPH model the free radical scavenging activity of green tea and fennel was measured by DPPH method with some modification. The reaction mixture of synthesized compounds at different concentration aliquots was taken and the volume was adjusted up to 3 mL with methanol. To this mixture 1 mL of 0.1 mM solution of DPPH in methanol was added. The mixture was kept in the dark for 30 min. The free radical scavenging activity of synthesized compounds was compared with standard (Vit.C). One milliliter of 0.1 mM of methanolic solution of DPPH and 3 mL of methanol was considered as control.

$$\% \text{ of inhibition} = [(A_{cont} - A_{test})/ A_{cont}] \times 100$$

Where Acont is the absorbance of control and Atest is the absorbance of the test sample. The sample concentration providing 50% inhibition (IC50) was determined. All the experiments were carried out in triplicate and IC50 values were expressed as mean ± SD

**ABTS Test**

The study was carried out in accordance with a procedure that was previously reported by Re and colleagues. In the beginning, 0.36 g of ABTS was dissolved in some distilled water. The following stage is 0.0662 g. dissolved, a solution of potassium persulfate (K2S2O8) was added, and the mixture was filled. Up to a final volume of 100 mL with water. To create the ABTS radical cation (ABTS+), the mixture was allowed to sit at room temperature in the dark for 16 hours. After then, ethanol was used to dilute the solution 100 times. Water was added to green tea infusions and fennel to dilute them (1 + 3 v/v). 10 L of the samples were then 1 mL of ABTS+ was added to the mixture, which was then incubated at room temperature in the dark for 6 minutes to measure the absorbance at a wavelength of 736 nm. The antioxidant capacity was demonstrated. Employing Trolox-like substances. Several Trolox concentrations in water were created for this purpose. (0–15 mM/L) and handled in the same manner as the samples under investigation.

**Experimental Design**

Twenty five adult male albino rats, weighting (150±5g), were used in this study. The animals were put in separate cages under 50-60% humidity and were fed on basal diet and water was provided to the rats by means of glass tube projecting through the cage wire. The basal diet was prepared from fine ingredients (100 g) according to (**Reeves *et al.*, 1993**)**.** Rats were divided in five groups with 5 animals in each group. One group served as a normal control (Group1), the second group (20 rats) was injected with freshly prepared Alloxan in saline at a dose level of 150 mg/ kg body weight **(Lazarow & Palay, 1954, Wohaieb & Godin, 1987& Kakkar, *et al.*, 1998)**.** After all rats with FBG >126 mg/dl were considered to be obesity, they were divided into five groups as follows: G 1; was considered as control positive and was fed on basal diet (300 gm diet),G 2; was fed on basal diet + (800 gm fats and 600 gm starch) to the end of the experiment, G3, was fed on basal diet + (800 gm fats and 600 gm starch +300 gm fennel), G 4; was fed on basal diet of + (800 gm fats and 600 gm starch +270 gm fennel + 30 gm green tea) and G 5; was fed on basal diet + ( 800 gm fats and 600 gm starch +240 fennel + 60 gm green tea). At the end of experiment, blood samples were collected for biochemical analyses.

***NO Assay***

The blood was collected in heparinized tubes. The liver samples were homogenized in cold 0.9% saline. The homogenates were then centrifuged at 10,000 r/min for 5 min at 4 °C; the supernatant was taken for NO assay and total protein determination. Nitric oxide production was determined by measuring in serum samples and liver homogenates total

NOx, the stable end products, using a modification of Griess's reaction, as previously described (**Xu *et al.*, 2011**).

***Protein Assay***

Homogenates of liver tissue were examined for estimation of total protein per well using the Bio-Rad protein micro assay. The protein assay was based on Bradford's dye-binding procedure (**Martin *et al.*, 2008**). Briefly, known concentrations of bovine serum albumin were used as standard curves. Two hundred microliters of sample or standard and 50 µL of Bio-Rad protein assay was added per well in a 96-well microtiter plate and protein was measured with a microplate reader.

***CT Images***

The micro-CT system, which has been extensively discussed previously (**Badea *et al.*, 2004**), rotates the animal that is held vertically while using a stationary tube and an X-ray detector. This allows for the use of a high flux rotating anode X-ray source (Philips SRO 09 50) with a dual 0.3/1.0 mm focal point. To reduce the motion blur from the heart, exposures as brief as 10 msec are supported by the system's flux. The 1.0-mm focused spot, for instance, can sustain 50 kW, which is more than 6000 times the power of conventional micro focal spot X-ray tubes. The geometric blur can be reduced by using the stationary tube/detector with the revolving specimen, leading to a net flux increase at the detector of almost 250 over commercial systems (**Badea *et al.*, 2004**). We used a high-resolution detector with 50 50 mm pixels covering a 2048 × 2048 picture matrix with a 106 mm active area input (Micro photonics X-ray Image Star camera, East Sussex, UK). We made use of a hardware feature that decreased the effective detector pitch to 100 mm by binning pixels into a 22 array.

**Cytotoxic activity**

*Human tumor cell lines*

Human tumor carcinoma cell lines (Liver carcinoma cell line {HEPG2} and Lung carcinoma cell line {HCT} used in this study were obtained from the National Cancer Institute (UICC), Cairo, Egypt, by serial sub-culturing.

*Drug*

Samples were prepared by dissolving 1:1 Stock solution and stored at -20◦C in dimethylsulfoxide (DMSO) at 100 mM. Different concentrations of the drug were used 10% (fennel only, fennel: green tea 9:1 and fennel: green tea 4:1) µg/ml.

*Cells and culture conditions*

RPMI-1640 medium was used for culturing and maintenance of the human tumor cell lines then the medium was supplied in a powder form and the working solution was prepared by dissolving 10.4 gm powder

and 2 gm sodium bicarbonate dissolved in 1 L distilled water. The medium was sterilized by filtration in a Millipore bacterial filter (0.22 µm). The prepared medium was kept in a refrigerator (4 0C). Before use the medium was warmed at 37 0C in a water bath and the supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum. Trypan blue dye: 0.05 % of the dye was prepared and used for viability counting. Fetal Bovine Serum (FBS): 10 % concentration was prepared and used also for supplementation of RPMI1640 medium prior to use. Penicillin/ Streptomycin: 100 units/ ml Penicillin/2 mg/ml Streptomycin were used for the supplementation of RPMI-1640 medium prior to use. Trypsin-EDTA: 0.25 % solution containing 2.5 g Pocrine trypsin was used for the harvesting of cells. A cryotube containing frozen cells was taken out of the liquid nitrogen container and then thawed in a water bath at 37oC.The cryotube was opened under strict aseptic conditions and its contents were supplied by 5 ml supplemented medium drop by drop in a 50 ml sterile falcon tubes. The tube was incubated for 2 hours then centrifuged at 1200 rpm for 10 minutes and the supernatant was discarded. The supernatant was discarded and the cell pellet was suspended and Seeded in 5 ml supplemented medium in T25 Nunclon sterile tissue culture flasks. The cell suspension was incubated and followed up daily the supplemented medium was replaced every 2- 3 days. Incubation was continued until a confluent growth was achieved and the cells were freshly sub cultured before each experiment to be in the exponential phase of growth. The medium was discarded. The monolayer cell was washed twice with 5 ml phosphate buffered saline. All the adherent cells were dispersed from their monolayer by the addition of 1 ml trypsin solution (0.25 % trypsin w/v) for 2 minutes.50 µl of 0.05 % trypan blue solution was added to 50 µl of the single cell suspension. The cells were examined under the inverted microscope using the haemocytometer. Non stained (viable) cells were counted and the following equation was used to calculate the cell count /ml of cell suspension.

Viable cells /ml = number of cells in 4 quarters X 2 (dilution factor) X 104

The cells were then diluted to give the required cell number for each experiment. To avoid the loss of the cell line, excess cells were preserved in liquid nitrogen as follows: Equal parts of the cell suspension and freezing medium (10 % DMSO in supplemented medium) were dispersed to cryotubes. The cryotubes were racked in appropriately labeled polystyrene boxes, gradually cooled till reaching -80 oC. Then the cryotubes were stored in a liquid nitrogen (-180oC) till use.

*Determination of potential cytotoxicity of drug on human cancer cell line*

*Principle*

The cytotoxicity was carried out using Sulphorhodamine-B (SRB) assay following the method. Nat. Protoc. 2006:1, 1112-1116. SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content (**Vichai and Kirtikara 2006)**.

*Reagants and buffers*

Glacial acetic acid: 1 % was used for dissolving the unbound SRB dye. Sulphorhodamine-B (SRB): 0.4 % concentration was dissolved in 1 % acetic acid was used as a protein dye. Trichloroacetic acid (TCA): 50 % stock solution was prepared, 10 % solution was used for protein precipitation. Tris base, 10 mM, (pH 10.5) was used for SRB dye solubilization. It was prepared by dissolving 121.1 gm of tris base in 1000 ml distilled water and pH was adjusted by 2 M HCl.

*Procedure*

Cells were seeded in 96-well microtiter plates at initial concentration of $3x10^3$ cell/well in a 150 µl fresh medium and left for 24 hours to attach to the plates. Different concentrations 10% (fennel only, fennel: green tea 9:1 and fennel: green tea 4:1) µg/ml of drug were added. For each drug concentration, 3 wells were used. The plates were incubated for 48 hours. The cells were fixed with 50 µl cold trichloroacetic acid 10% final concentration for 1 hour at 4 $^0$C.The plates were washed with distilled water using (automatic washer Tecan, Germany) and stained with 50 µl 0.4 % SRB dissolved in 1 % acetic acid for 30 minutes at room temperature. The plates were washed with 1 % acetic acid and air-dried. The dye was solubilized with 100 µl/well of 10M tris base (pH 10.5) and optical density (O.D.) of each well was measured spectrophotometrically at 570 nm with an ELISA microplate reader (Sunrise Tecan reader, Germany). The mean background absorbance was automatically subtracted and means values of each drug concentration was calculated. The experiment was repeated 3 times.

*Calculation*

The $IC_{50}$ values (the concentrations of resveratrol required to produce 50% inhibition of cell growth) were also calculated.

**Results and discussion**

***Phytochemical study***

All results of phytochemical analysis are showed in Table 1&2. In the present study, the green tea extract and fennel extract showed positive results for triterpenes and/or steroids as measured by the Liebermann-Burchard reaction. It was found that both of extracts contained polyphenols and flavonoids, which may be responsible for the biological activities found.

**Table 1.** Phytochemical screening of green tea extract. Highly positive '++++', Moderate '++',

| Test | Steroids | Terpenoids | Flavonoids | Alkaloids | Saponins | Tannins |
|---|---|---|---|---|---|---|
| **Result** | ++ | +++ | ++++ | ++ | +++ | ++ |

**Table 2.** Phytochemical screening of fennel extract. Highly positive '++++', Moderate '++',

| Test | Steroids | Terpenoids | Flavonoids | Alkaloids | Saponins | Tannins |
|---|---|---|---|---|---|---|
| **Result** | ++ | +++ | ++++ | ++ | +++ | ++ |

***Chemical Composition***

All solvents and chemicals were from Sigma-Aldrich St. Louis, MO, USA. The international standards use different methods and parameters to evaluate the quality of the plants according their useas food. Physicochemical parameters were considered in relation to the requirements of standards.

Chemical composition of the investigated green tea was presented in (**Table 3**). Every 100 g edible portion of green tea seeds contain on average: 21.5 g protein; 2.3 g fat; 12.4 g fibre; 8.17 g humidity and 1.6 g ash contents were tested.

**Table 3:** Chemical Composition of Green tea

| Measuring | Result | + ; - | Method used in measuring |
|---|---|---|---|
| **Protein** | 21.5 | 0.82 | ES: 5465-1/2006 |
| **Fat** | 2.3 | 0.05 | |
| **Fiber** | 12.4 | 0.93 | EN (EC)NO.152/2009 |
| **Humidity** | 8.17 | 0.15 | |
| **Ash** | 6.1 | 0.06 | ES: 5464/2006 |

The chemical composition of the investigated fennel varies with morph type, source, climate and harvesting stage. Every 100 g edible portion of fennel seeds contain on average: 18.9 g protein; 8.6 g fat; 27.7 g fibre; 9.92 g humidity and 8.6 g ash contents were tested (**Table 4**).

**Table 4:** Chemical Composition of fennel

| Measuring | Result | + ; - | Method used in measuring |
|---|---|---|---|
| **Protein** | 18.9 | 0.72 | ES: 5465-1/2006 |
| **Fat** | 8.6 | 0.24 | |
| **Fiber** | 27.7 | 2.06 | EN (EC) NO.152/2009 |
| **Humidity** | 9.92 | 0.18 | |
| **Ash** | 6.8 | 0.06 | ES: 5464/2006 |

***Antioxidant screening***

In general, phenolic compounds and nitrogen-bearing heterocyclic rings have free radical scavenging activity. DPPH radicals accept the hydrogen atom or electron from the organic molecules and can form stable diamagnetic molecules. Scavenging effect of green tea extract showed 50% of inhibition ($IC_{50}$) at a concentration level of 19.64±0.13µg/mL, also fennel extract showed at 54.56±0.32 while standard Vit.C showed at 16.81±0.10µg/mL (Table 4). But in case of ABTS model Scavenging

effect of green tea extract showed 50% of inhibition ($IC_{50}$) at a concentration level of 33.47±0.21µg/mL, also fennel extract showed at 67.15±0.39 while standard Vit.C showed at 29.47±0.17µg/mL (Table 5). These results prove that the green tea and fennel are obviously good-excellent antioxidants (i.e., effective anti-DPPH• compounds). Being very close and relatively different, the differences in values in this assay can be explained by and attributed to the same effects of structural modifications (i.e., differences) that were previously mentioned under ABTS test.

**Table 5:** Antioxidant activity of the green tea and fennel using the DPPH test.

| Comp. | Conc (µg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10 | 20 | 40 | 60 | 80 | 100 | $IC_{50}$ |
| | % Inhibition | | | | | | |
| Vit.C | 38.7 | 52.1 | 69.6 | 81.8 | 87.4 | 94.5 | 16.81±0.10 |
| Green tea | 32.6 | 50.0 | 64.8 | 78.3 | 91.2 | 97.8 | 19.64±0.13 |
| Fennel | 14.5 | 25.3 | 36.1 | 48.4 | 62.7 | 72.9 | 54.56±0.32 |

**Table 6:** Antioxidant activity of the green tea and fennel using the ABTS test.

| Comp. | Conc (µg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10 | 20 | 40 | 60 | 80 | 100 | IC50 |
| | % Inhibition | | | | | | |
| Vit.C | 24.2 | 37.4 | 54.9 | 66.7 | 78.5 | 94.3 | 29.47±0.17 |
| Green tea | 21.5 | 36.5 | 50.1 | 64.5 | 71.0 | 89.5 | 33.47±0.21 |
| Fennel | 11.8 | 23.6 | 32.7 | 43.1 | 56.2 | 64.0 | 67.15±0.39 |

## Estimation of Nitric oxide (NO) level in Serum

An effect of fennel and green tea on fed on bad diet –induced NO production, however there were no *in vivo* animal studies examining the effect role of fennel and green tea on Nitric oxide production. Thus, we designed the following animal experiments to study the effect of fennel and green tea. In each of the ten groups, the rats increased their weight at a constant rate. There were no differences in weight gain among the groups (data not shown). NO production was estimated in serum from rats of G1 that served as controls by measuring the stable metabolite NO. A normal production of NO (13.41 ± 0.84 µM) was found in these samples. Fennel and green tea mixture was found to induce a significant increase of NO in serum (G3–G5 in Figure 1). Among the groups of the rats fed on fennel and green tea 9:1 (G4; 16.21 ± 0.54 µM) slightly reduced the NO level in serum, compared with that, group fed on fennel and green tea 4:1 (G5; 14.14 ± 0.81 µM). See table 7 and figure 1. These results indicated that fennel and green tea 4:1 feeding can increase serum NO levels. These results were evidence by axial CT scan for the blood flow in the blood vessels of rats of both +Ve control which fed on diet with high lipid and polysaccharide content and group 5 which, fed on basal diet + (800 gm fats and 600 gm starch using mixture fennel - green tea with ratio 4:1)

which showed that the mixture of fennel with green tea as ratio 4:1 enhance the pass flow of blood in blood vessels which clear in the figure 1 as spot light.

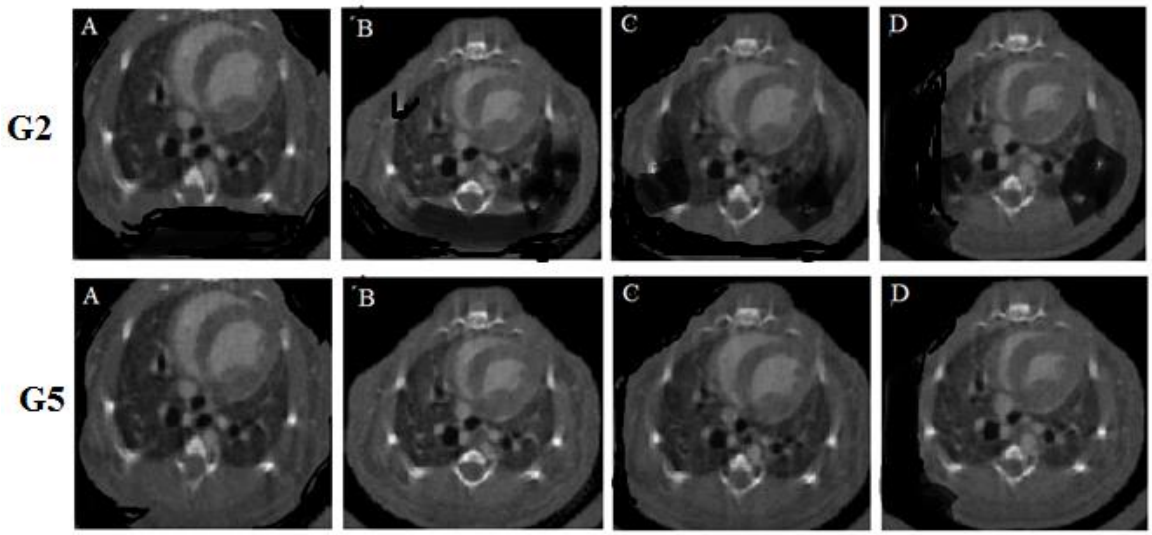


**Figure 1.** CT of the mouse heart during four phases of the cardiac cycle at (A) 0, (B) 10, (C) 15 and (D) 30 msec after the R peak for groups 2 &5.

**Estimation of Nitric oxide (NO) level in Liver**

In liver homogenates of the rats, the metabolic level of NO was shown as G1 in Figure 2. A basal production of NO was about 5.00 ± 0.11 µmol/µg of protein in liver. In the groups of rats that were administered as controls. NO production in the liver was slightly inhibited by being fed with high fat and polysaccharides in G2, 0.9 ± 0.18 µmol/µg. In Figure 2. Fennel and green tea mixture was found to induce a significant increase of NO in liver (G3–G5 in Figure 1). Among the groups of the rats fed on fennel and green tea 9:1 (G4; 8.91 ± 0.11 µmol/µg) slightly reduced the NO level in serum, compared with that, group fed on fennel and green tea 4:1 (G5; 6.16 ± 0.85 µmol/µg). These results indicated that fennel and green tea 4:1 feeding can increase liver NO levels.

**Table 7:** Nitric oxide productions in rat serum and liver.

| Parameters / Groups | NO Level in Blood | NO Level in Liver |
|---|---|---|
| **($G_1$) Control (-)** | 13.41 ± 0.84 | 5.00 ± 0.11 |
| **($G_2$) Control (+)** | 2.18 ± 0.73 | 0.90 ± 0.18 |
| **($G_3$) fennel** | 19.22 ± 0.71 | 9.71 ± 0.21 |
| **($G_4$) 9:1** | 16.21 ± 0.54 | 8.91 ± 0.11 |

| | | |
|---|---|---|
| **($G_5$ ) 4:1** | 14.14 ± 0.81 | 6.16 ± 0.85 |

***Cytotoxic activity***

Almost of the cancer treatments are highly associated with herbal supplements. There is beneficial effect of medicinal plants on cancer. As herbal remedies are used to improve the quality of life. Plant derived natural products such as flavonoids, terpenes, alkaloids etc. have received great attention in recent years due to their various medicinal properties including cytotoxic and cancer chemo preventive effects (Babu et al., 2002).

Research on anti-cancer agents from plant sources started in the 1950s with the discovery and development of the vinca alkaloids like vincristine and vinblastine, and the isolate toxins from cells. Natural products discovered from medicinal plants have played a vital role in cancer protection. Natural products or natural product derivatives consisting of 14 of top 35 drugs in 2000 on the basis of global sales (Butlet MS., 2004). Plant based medication has found a role in cancer healing (chemotherapy), and the mechanism of interaction between many phytochemicals and cancer cells has been widely studied .There is an increasing interest in herbal medicines in particular estimation of various plants used in Indian tradition system . There are more than 2, 70,000 higher plants existing on this planet. So, it is expected that plants can provide

Potential bioactive compounds for the development combat cancer diseases (Shoeb M., 2006). The phytochemical screening of HEBE showed presence of chemical compounds such as alkaloids, flavonoids, glycosides, phenolics, steroids, tannins and terpenoids, except saponins. Medicinal plants may contain various kinds of chemical components and their biological activities (Cho et al., 2003). The present study was undertaken to assess the cytotoxic activity of liver the cytotoxic result obtained in the present study demonstrates for the first time. The cytotoxicity was measured by concentration that caused 50% loss of cell monolayer ($IC_{50}$) .Fennels and its mixtures with green tea were tested against HepG2 (human hepatocellular liver carcinoma cell lines) and VERO (cell lines were initiated from the kidney of a normal adult African green monkey) as normal cell. The fennel and its mixtures with green tea (GT) as ratios 9:1 and 4:1 respectively. Obtained results showed fennel with green tea as ratio 4:1 have a significant activity as antitumor agents against the tested human hepatocellular liver carcinoma cell lines without effect on normal cells. Table 8

**Table 8:** Cytotoxicity ($IC_{50}$) of tested fennel and its mixtures on different cell lines.

| Samples | HepG2 | | | | VERO | | | |
|---|---|---|---|---|---|---|---|---|
| | **Sample conc. (µg/ml)** | | | | | | | |
| | **125** | **250** | **500** | **1000** | **125** | **250** | **500** | **1000** |
| | **Viability %** | | | | | | | |
| **Fennel** | 65.11± 0.11 | 56.11± 2.01 | 49.11± 1.16 | 48.11± 0.11 | 95.43± 3.32 | 95.34± 0.11 | 91.11 ±1.41 | 85.12± 0.01 |
| **$IC_{50}$** | 32.83±9.8 | | | | 92.13±2.1 | | | |
| **Fennel : GT 9:1** | 55.01± 1.12 | 51.12± 1.32 | 45.01± 1.11 | 21.01± 1.12 | 95.11± 1.14 | 85.11± 1.12 | 85.01 ±1.22 | 79.11± 1.14 |
| **$IC_{50}$** | 22.83±9.8 | | | | 88.11±1.1 | | | |
| **Fennel : GT 4:1** | 41.91± 0.18 | 37.21± 1.11 | 21.91± 0.18 | 09.91± 0.11 | 91.41± 1.12 | 81.21± 0.18 | 80.91 ±0.12 | 71.91± 1.18 |
| **$IC_{50}$** | 8.81±1.8 | | | | 82.11±0.1 | | | |

## Conculsion

The Review's Objective This review looks at the importance of nitric oxide (NO) and summarizes the studies on the link between cognitive decline and cardiovascular disease (CVD). The (NO) pathway is one of the possible causes. Recent findings there are evidence from epidemiological research that midlife CVD and associated risk factors increase the likelihood of dementia and cognitive impairment in later life. The exact source of this link is unknown, but risk may be communicated by an increase in vascular alterations, such as small artery disease, neurodegeneration (such as amyloid deposition), and changes in NO bioavailability. 9:1 (G4; 16.21) among the rat groups fed fennel and green tea. According to the results, fennel and green tea, in a ratio of 4:1, significantly inhibited the growth of the tested human hepatocellular liver cancer cell lines while having no effect on healthy cells. The phenolic components of traditional medicinal herbs linked to antioxidant action are characterized in this work. The total antioxidant capacity was thoroughly evaluated using the enhanced ABTS•+ DPPH technique.

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**ملخص البحث:**

هدف الدراسة ، قمنا بفحص أهمية أكسيد النيتريك (NO) وتلخيص البحث حول العلاقة بين أمراض القلب والأوعية الدموية (CVD) والضعف الإدراكي. الطريق (لا) هو نمط من السببية. الاكتشافات الحديثة وجدت الدراسات الوبائية أدلة تربط بين الأمراض القلبية الوعائية في منتصف العمر وعوامل الخطر ذات الصلة بزيادة خطر الإصابة بالخرف والتدهور المعرفي في وقت لاحق من الحياة. سبب هذا الارتباط غير معروف ، ولكن قد يتم الإبلاغ عن المخاطر من خلال زيادة التنكس العصبي (مثل ترسب الأميلويد) ، وتغيرات الأوعية الدموية (مثل مرض الأوعية الدموية الصغيرة) ، وآليًا بسبب زيادة مستويات الإجهاد التأكسدي والالتهاب وكذلك تغييرات في التوافر البيولوجي. من بين مجموعات الفئران التي تتغذى على الشمر والشاي الأخضر ٩: ١ (G4 ؛ ١٦.٢١ ± ٠.٥٤ ميكرومتر) خفضت بشكل طفيف مستوى NO في مصل الدم ، مقارنةً بالمجموعة التي تتغذى على الشمر والشاي الأخضر ٤: ١ (G5 ؛ ١٤.١٤ ± ٠.٨١ ميكرومتر). أظهرت النتائج التي تم الحصول عليها أن الشمر مع الشاي الأخضر بنسبة ٤: ١ له نشاط كبير كعوامل مضادة للأورام ضد خطوط خلايا سرطان الكبد البشرية المختبرة دون تأثير على الخلايا السليمة. تميز هذه الدراسة نشاط مضادات الأكسدة والمركبات الفينولية للنباتات الطبية التقليدية المرتبطة بمضادات الأكسدة ، وقد تم استخدام طريقة ABTS + DPPH • المحسنة لتقييم القدرة الكلية المضادة للأكسدة بشكل منهجي.