The Promising Anticancer Efficacy of Parsley Seeds Flavonoid (Apigenin) in Induced Mammary Adenocarcinoma (AMN3) Mice

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Abstract

Extraction and identification of parsley *(Petroselinum sativum*) seeds flavonoids (apigenin), as well as evaluation its anticancer efficacy was the main aim of the current study. Thin layer chromatography results clarified that apigenin is the major flavonoid in parsley seeds. The cytotoxic effect of apigenin in mammary adenocarcinoma (AMN3) bearing mice was manifested through significant ($P \leq 0.01$) reduction in tumor volume and growth rate inhibition (90.8 %) after 24 days of oral administration at a dose of 300 mg/kg body weight. The volume of tumor in the treated group reached 1354.8 mm^3 while the recorded size of the control was 14758 mm^3. Transplanted cancer mice showed a significant ($P \leq 0.01$) elevation in concentration of liver, heart, kidney and tumor mass tissue homogenate malondialdehyde as well as a significant depression in glutathione concentration. Apigenin intubation caused a significant correction of the previous parameters manifested by a significant elevation and depression in glutathione and malondialdehyde concentration respectively. Agarose gel electrophoresis of blood serum of AMN3 transplanted mice, showed an increment in globulin concentrations coincide with albumin concentration decrement, while apigenin administration normalized to a great extent serum protein concentrations. Histopathological sections of liver, kidney, spleen as well as tumor mass showed the significant role of apigenin in immune system stimulation manifested by elevation of globulins, lymphocytes and macrophages and replacement of cancer tissues by connective tissues.

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Flavonoids are phenolic compounds which are widely distributed in plants, and have been reported to exert multiple biological effects, including antioxidant, free radicals scavenging abilities anti-inflammatory and anticarcinogenic activity.[1,2,3] The flavonoid apigenin is regarded as the active principle of parsley and is known to possess an antioxidant property and effectively militates against the pro-oxidative activity of cadmium.[4,5,6] In addition, apigenin showed anticancer effect against lung cancer[7] as well as, growth inhibition of human colon carcinoma cell lines.[8] Human trials on the antioxidant effects of beverages rich in polyphenolics, such as red wine, fruit juice or tea and vegetables rich in flavonoid like parsley, have been limited and results are, at present, inconclusive, in part, to poor methodologies available to measure oxidative damage in vivo.[9] Therefore, this study was performed in normal and mammary adenocarcinoma (AMN3) bearing mice to investigate 1) the effect of parsley seeds apigenin on the growth of transplanting tumor in mice, 2) lipid peroxidation (glutathione and malon-dialdehyde concentration) and serum protein profile in normal and treated animals, and 3) histopatho-logical changes of liver, kidney and spleen as well as tumor mass in normal and treated mice.

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Materials and Methods

Flavonoid extraction and identification

Extraction of flavonoids from parsley seeds was carried out according to Harbone method[10] modified by Al-Kawary, 2000.[11] Acid hydrolysis was carried out by 2N hydrochloric acid and, ethyl acetate and petroleum ether as organic solvent.

Identification of flavonoids by thin layer chromatography[12] on silica gel plates was conducted using, n- butanol : acetic acid : water (40:10:50) as a mobile phase, UV detector was used to explore the spot at 245 nm.

Immune-suppressed animals

Preparation of immune-suppressed mice was carried out by dividing a group of 70 animals into two groups:

In the first group , fifty mice were administered orally 10 mg/kg body weight (BW) of levamisole in 3 days intervals, plus 0.5 % H_2O_2 in drinking water for one month.[13]

In the second group, twenty mice were administered orally 50 mg/kg of cyclosporine daily (Novartis Pharma., France) until the end of the experiment. Drinking water and food pellets were sterilized for 20 and 10 minutes respectively.[14] The results showed that, levamisole plus H_2O_2-treated mice were found to be infected by mammary adenocarcinoma while cyclosporine-treated group was not.

Transplantation of tumor cells in the immune-suppressed mice

Mammary adenocarcinoma bearing mouse, from the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR), was used as a source for tumor cells transplantation into immune-suppressed adult females albino mice supplied from ICCMGR.

Anticancer efficacy of apigenin *in vivo*

Group 1: Five mice with documented mammary adenocarcinoma were administered a daily dose of 300 mg/kg BW of apigenin up to the end of the experimental period (24 days). Administered dose *was* selected according to the result of the cytotoxicity test *in vitro*.[15]

Group 2: Five mice carrying mammary adenocarcinoma received only distilled water and considered positive control for the first group.

Group 3: A group of ten normal mice were administered apigenin orally, a daily dose of 300 mg /kg BW for 30 days.

Group 4: Ten normal mice received distilled water for 30 days to serve as a control group for Group 3.

Group 5: Forty mice represented a group which resisted AMN3.

Measurement of tissue glutathione[16]

Homogenized tissues (liver, spleen, kidney, tumor mass) were prepared in Tris buffer (50 mM Tris, 0.1 mM EDTA, pH 7.6), 25% trichloroacetic acid solution, 0.15 M imidazole (pH 7.4), 3 mM DTNB (5, 5-dithiobis 2-nitrobenzoic acid) freshly prepared in imidazole buffer. The optical density of the sample was measured spectrophotometrically at 412 nm and glutathione concentration was expressed as μmol/g wet tissue.

Measurement of tissue malondialdehyde[17]

Homogenated tissues (liver, spleen, kidney, tumor mass) were prepared in sodium chloride (0.9 % NaCl)-2 mM NaN_3 (sodium azide), trichloroacetic acid (TCA 28%)-0.1 M Na-arsenite, thiobarbituric acid 1% in 0.05 M NaOH (freshly prepared; heating required), Tris (50 mM)-0.1 mM EDTA pH 7.6.

Hydrogen peroxide 30 % (11 μl 30 % H_2O_2 + 10 ml saline azide) was freshly prepared. The absorbance was measured at 532 nm and 453 nm using 1.53×10^5 M^{-1} as molar extinction coefficient and the values were expressed as nmol TBA RS/g wet tissue.

$$\text{Tissue MDA} = \Delta A / (1.53 \times 10^5)$$

[ΔA = (Abs. sample 532 - blank 532) - 20% (Abs. sample 453 - blank453)]

Agarose gel electrophoresis

Electrophoretic separation of serum proteins of different treated groups according to their relative mobility on agarose gel was carried out using Hellabio agarose gel kit (Greece). Serum protein fractions were fixed and stained by amido black and the percent protein fractions were estimated by Hellabioscan or densitometer at 520 nm.

Measurement of Tumor volume[18]

Tumor volume, was measured in three days intervals up to the end of experimental period (24 days) using vernier calipers, volume of the tumor was calculated as follows

$$\text{Tumor volume } (mm^3) = a \times b^2 / 2;$$

where a = tumor length and b = tumor width.

Measurement of percentage of tumor inhibition[19]

Following tumor volume calculation, inhibition in the rate of tumor growth (GI) was carried out according to the equation:

$$GI\% = 100 \times (A - B) / A;$$

where GI = growth inhibition. A = volume of untreated tumor, B = volume of treated tumor.

Histopathological test

For histological studies, organs in five treated animals (liver, spleen, kidney and tumor mass) were extracted and preserved in 10% formalin until the preparation of histopathological sections.

Statistical analysis

Data were analyzed statistically by using one way analysis of variance (ANOVA) for tissue glutathione (GSH) and malondialdehyde (MDA) determination.[20]

Results

Extraction and identification of flavonoids from *Petroselineum sativum* seeds

The result of this study revealed that out of each kilogram of *Petroselineum sativum* dry seeds, approximately 2.69 g crude flavonoids were obtained. Apigenin appeared as a dominant flavonoid on silica gel plates with Rf value of 0.81 (Figure 1).

Treatment of tumor by using apigenin

Daily administration of 300 mg/kg BW of apigenin to a group of mice with induced mammary adenocarcinoma caused significant ($P \leq 0.01$) percent inhibition (90.81 %, Table 1) in tumor volume after 24 days of treatment as compared to zero time. Figure 3A and 3B confirmed clearly the significant reduction in tumor volume of treated (Figure 3B) and untreated (Figure 3A) animals.

Malondialdehyde (MDA) and glutathione (GSH)

Malondialdehyde (nmol/g) and glutathione (μmol/g) concentration in heart, liver , kidney and tumor mass homogenates wet tissues of normal mice, mice with

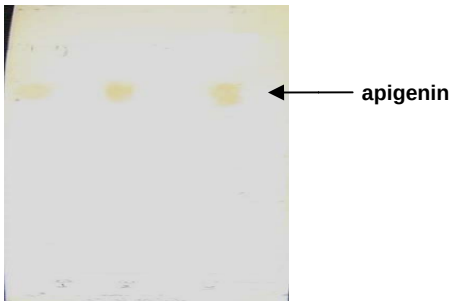

Figure 1 Chromatogram TLC analysis using butanol: acetic acid: water as mobile phase; three different dilutions of flavonoid (apigenin) in ethyl acetate are shown.

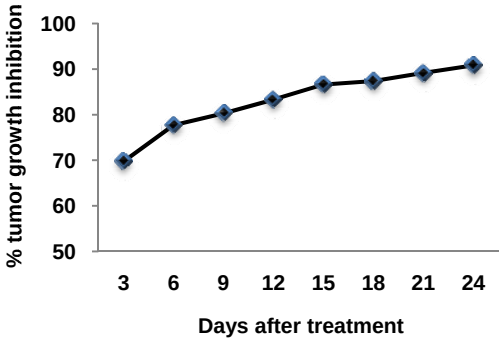

Figure 2 Percentage of tumor growth inhibition in treated group (mice received 300 mg/kg apigenin orally for 24 days); n = 5; $P \leq 0.01$.

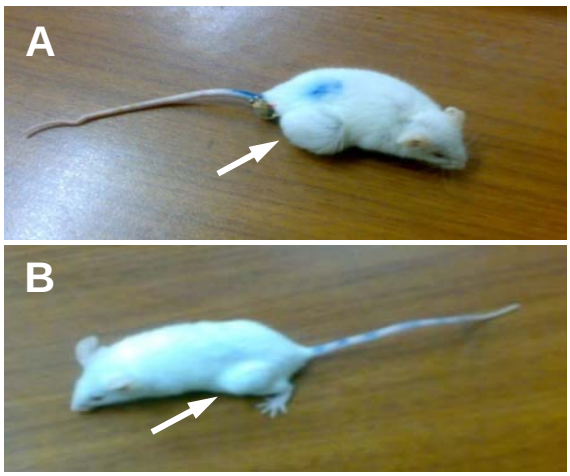

Figure 3 Comparing the tumor size in mouse bearing mammary adenocarcinoma; *A*, untreated (Group 2), receiving distilled water orally for 24 d; and *B*, treated (Group 1), receiving 300mg/kg of apigenin orally for 24 d.

AMN3 cancer and mice with AMN3 treated with apigenin, were shown in Table 2 and 3. The results showed significant elevation and depression ($P \leq 0.01$) in malondialdehyde and glutathione concentration in mammary adenocarcinoma-bearing mice organs and tumor mass homogenates (Table 2 and 3), respectively, as compared to the control, while oral administration of 300 mg/kg BW of apigenin to mice with AMN3 caused significant normalization of those values ($P \leq 0.01$).

Table 1 Comparison of tumor volume (mean ± SEM) and relative size (in % reduction) in apigenin-treated and untreated (Group 2 and Group 1) mice with induced mammary adenocarcinoma.

Days of treatment	Tumor volume (mm^3)		% reduction
	Untreated (Group2)	Treated (Group 1)	
3	1,587±1.25^{h}	480.5±2.04^{g}	69.72^{d}
6	4,284.6±2.45^{g}	956.5±2.45^{f}	77.68^{c}
9	6,927.5±4.10^{f}	1,364±2.44^{d}	80.30^{b}
12	9,187.5±5.31^{e}	1,533±1.22^{b}	83.31^{b}
15	9,643.5±1.22^{d}	1,290±4.08^{e}	86.62^{a,b}
18	12,728.8±2.9^{c}	1,605±2.04^{a}	87.39^{a}
21	13,712±3.3^{b}	1,485.9±2.05^{c}	89.16^{a}
24	14,758±3.27^{a}	1,354.8±1.63^{c}	90.81^{a}

Group 1, mice with mammary adenocarcinoma receiving 300 mg/kg apigenin orally for 24 d; Group 2, mice with mammary adenocarcinoma received distilled water orally for 24 d; superscripted alphabets denote significance at $P \leq 0.01$; number of mice = 5.

Table 2 Malondialdehyde concentration (nmol/g wet tissue)

Groups	Heart	Liver	Kidney	Tumor
Con (-ve)	0.49±0.02^{c}	0.69±0.03^{c}	0.33±.003^{c}	-
Con (+ve)	1.37±0.05^{a}	2.66±0.06^{a}	0.46±0.17^{a}	2.16±0.17^{a}
Treated	0.60±0.02^{b}	1.57±0.06^{b}	0.40±.002^{b}	1.08±0.13^{b}

Con (-ve), or Group 3, normal mice receiving distilled water for 30 d; Con (+ve), or Group 2, mice bearing mammary adenocarcinoma receiving distilled water for 24 d; Treated, or Group 1, mice bearing mammary adenocarcinoma receiving 300 mg/kg apigenin for 24 d; superscripted alphabets denote significance at P ≤ 0.01; number of mice = 5

Table 3 Glutathione concentration (µmol/g wet tissue)

Groups	Heart	Liver	Kidney	Tumor
Con (-ve)	6.93±0.03^{b}	15.0±0.3^{b}	1.18±0.01^{a}	-
Con (+ve)	5.11± 0.06^{c}	12.6±0.23^{c}	0.63±0.62^{c}	0.75±0.02^{b}
Treated	7.71±0.02a	16.0±0.20^{a}	1.09±0.03^{b}	2.40±0.01^{a}

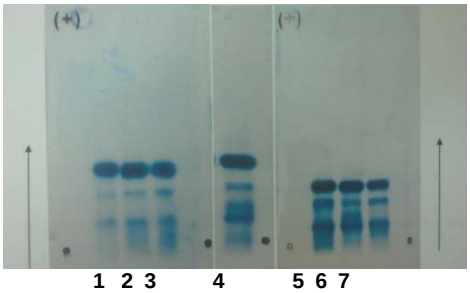

Figure 4 Agarose gel electrophoresis of blood serum at pH 8.6, size of sample 5 µl. ***1 & 6***, mice with mammary adenocarcinoma receiving 300 mg/kg apigenin orally for 24 d; ***2 & 3,*** normal mice receiving distilled water orally for 30 d; ***4***, mice inoculated with mammary adenocarcinoma but they were resistant; ***5***, normal mice receiving 300 mg/kg apigenin orally for 30 d; ***7***, mice with mammary adenocarcinoma receiving distilled water orally for 24 d.

Agarose gel electrophoresis

Changes in the concentration and patterns of the serum protein (albumin, α1-globulin, α2-globulin, β-globulin and γ-globulin) in normal and treated animals were shown in Table 4 and Figure 4.

The average albumin concentration in the serum of the mice (G2) was 38.84 % of the total protein, while the corresponding values in the control were

Table 4 Agarose protein electrophoresis (protein fraction, %)

Group	Albumin	Globulins			
		α1	α2	β	γ
1	57.74	11.85	6.33	15.07	8.99
2	38.84	10.98	2.92	42.8	4.44
3	33.87	20.83	5.23	24.0	16.05
4	59.95	7.14	1.61	15.89	15.39
5	47.67	9.71	15.67	16.40	10.53

Group 1, mice with mammary adenocarcinoma + 300 mg/kg apigenin orally for 24 d; *Group 2*, mice with mammary adenocarcinoma + distilled water orally for 24 d; *Group 3*, normal mice + 300 mg/ apigenin orally for 30 d, *Group 4,* normal mice + distilled water orally for 30 d; *Group 5*, mammary adenocarcinoma-resistant mice.

59.95 %. On the other hand a considerable elevation in albumin concentration was recorded in apigenin-treated group 57.74 % of the total protein as compared to the control, while normal mice received orally 300 mg/kg BW of flavonoid (apigenin) showed the lowest values 33.87 %. Moreover, albumin concentration in the group of the mice which showed endogenous resistances (failed cancer) was about 47.67 %. An increase in the level of α1- and α2-globulin was recorded in all treated groups as compared to the control which showed the lowest values 7.14 and 1.61 %, respectively. The corresponding values in mice with AMN3 cancer and apigenin-treated groups were 10.98 and 11.85 for α1-globulin, and 2.92 and 6.32 for α2-globulin, respectively. The highest concentration for β-globulin was recorded in mice with AMN3 cancer (42.8 %) and the lowest values in the control (15.89 %). Mice with AMN3 cancer administered with flavonoid (apigenin) showed a value of 15.07 %. Finally, γ-globulin fraction was lowest in mice with AMN3 cancer 4.44 % as compared with control 15.39 % and apigenin-treated group 8.99 %.

Histopathological study

Liver. Histopathological sections of liver in cancer-inoculated mice clearly showed cancer cell presence, as distinguished with hyperchromatin and pleomorphic in central veins and degenerative changes in liver cells with ruptured cell and cytoplasm (Figure 5A).

Spleen. Histopathological section of spleen in untreated cancer mice showed congestion of blood vessels and cancer cells in the cavity which was distinguished with hyperchromatin and pleomorphic, precipitation of amyloid in red pulp and wide spread of white pulp (Figure 6C), while histopathological examination of spleen in cancer, treated mice showed clarified precipitation of amyloid in spleen with hyperplasia of white pulp of cancer cells (Figure 6B).

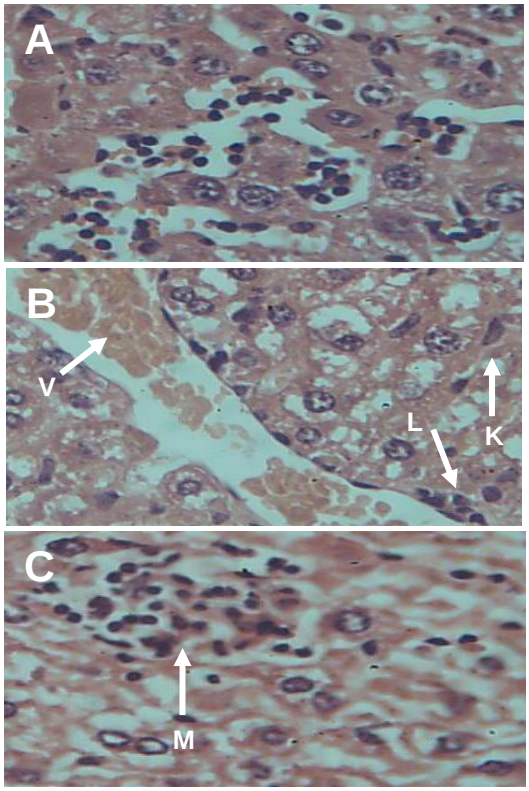

Figure 5 Liver histpathology. ***A***, Group 2 (cancer, untreated), 400x; arrow indicates macrophage-associated tumor cell in the sinusoid; ***B***, Group 1 (cancer, treated), 400x; K, Kupffer cell proliferation; V, congested central vein; L, lymphocytic aggregation around central vein; ***C***, Group 3 (normal, treated), 100x; showing the presence of kupffer cells and aggregation of monocyte (M).

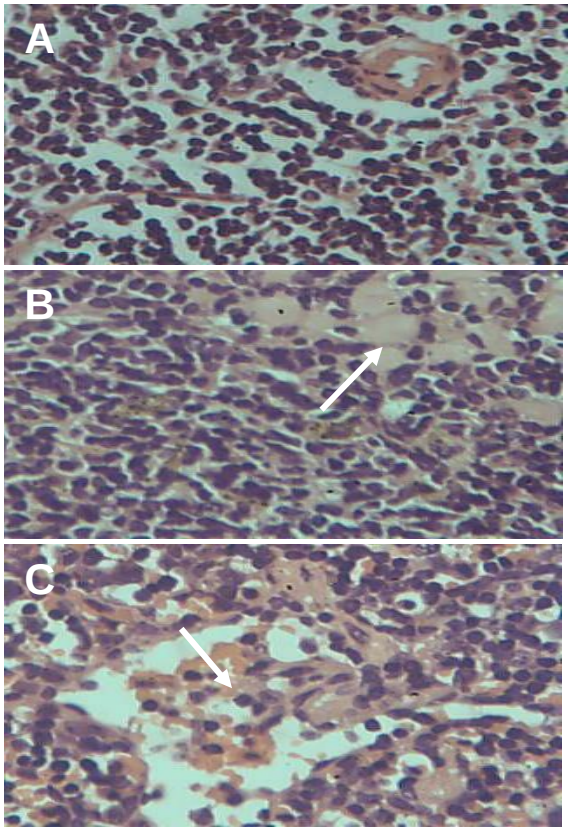

Figure 6 Spleen histopathology. ***A***, Group 3 (normal, treated), 100x; showing hypertrophy of medial layer of central artery with hyperplasia of periarterial sheet; ***B***, Group 1 (cancer, treated), 100x; arrow showing hyperplasia of white pulp with amyloid like substance deposition in the red pulp; ***C***, Group 2 (cancer, untreated), 400x; arrow showing congested red pulp with macrophage-associated tumor cells

On the other hand, spleen of normal mice received 300 mg/kg apigenin for 30 days showed hyperplasia of white pulp. Amyloid precipitate is not recorded in normal mice received apigenin, since cancer is the main cause of amyloid (Figure 6A).

Kidney. Kidney of untreated cancer mice showed histopathologically heavy growth of cancer cell around kidney glomeruli which lead to change in tissues (Figure 7A), while histopathological changes of kidney of treated cancer mice (300 mg/kg apigenin) showed congestion of blood vessels because of aggregation of lymphocyte and macrophage in parenchyma of kidney and there was no presence of cancer cells (Figure 7B) while section of kidney of normal mice treated with apigenin showed no pathological changes.

Tumor mass. Histopathological section of skin mammary adenocarcinoma mass, in untreated mice showed progressive stage of cancer, manifested by the presence of cancer cells with hyperchromatin and polymorphic in different size, with of proliferation of nucleus and cancer cell aggregation at glandular structure (Figure 9A), while histopathological section of tumor mass in mice treated with apigenin showed the presence of large necrotic area (necrotic cancer cells) surrounded with large amount of inflammatory cells like macrophage and lymphocyte with proliferation of connective tissue that replaced cancer cells (Figure 9B), while Figure 8 A showed macroscopic large tumor mass compared to treated mouse (Figure 8B). Results concerning histopathological changes were summarized in Table 5.

Discussion

The Rf value of apigenin recorded in this study was approximately the same as that recorded by Harbone, 1984**,** under similar diagnostic condition. In addition to parsley seeds, apple, beans, celery, cherries, cloves, grapes, onions, barley, tomatoes, represent major sources of food rich apigenin. Furthermore, plants-derived beverage, including tea and wine contained considerable amount of apigenin.[21]

Along the experimental period, mice were in good condition, active, with increasing food consumption, and all treated mice remain alive after treatment indicating the protective effect of apigenin[22] against cancer damage. Inhibition of another transcription factor like activator protein (AP) which leads to the inhibition of transcription and cascade of apoptosis[23] by apigenin could be another possible pathway. Besides, flavonoids inhibit Na^+-K^+ ATPase enzyme in the plasma membrane leading to increased intracellular Na^+ and Ca^{2+} concentrations, and decreased K^+ concentration, with libration of cytochrome c from mitochondria resulting in apoptosis.[24] Anticancer activity of apigenin may be attributed to the high inhibition of hypoxia inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) expression in the tumor tissue with

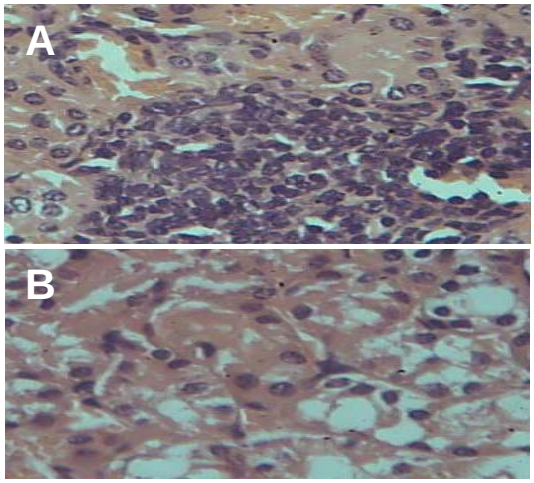

Figure 7 Kidney histopathology; ***A***, Group 2 (cancer, untreated); B, Group 1 (cancer, treated).

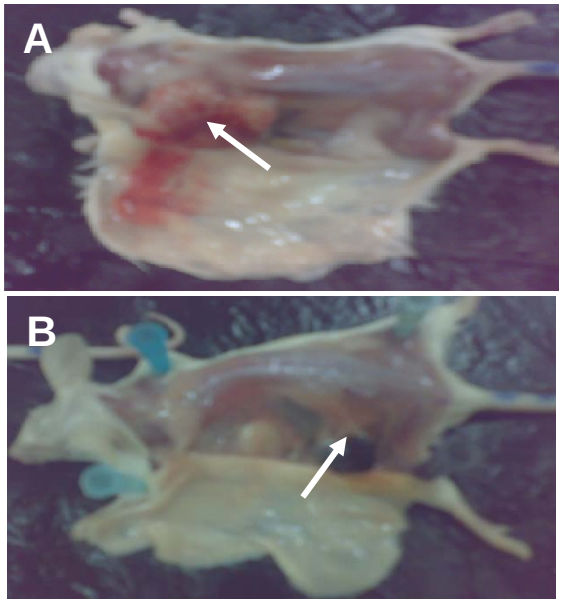

Figure 8 Tumor mass was large in ***A***, Group 2 (cancer, untreated), compared with ***B***, Group 1 (cancer, treated).

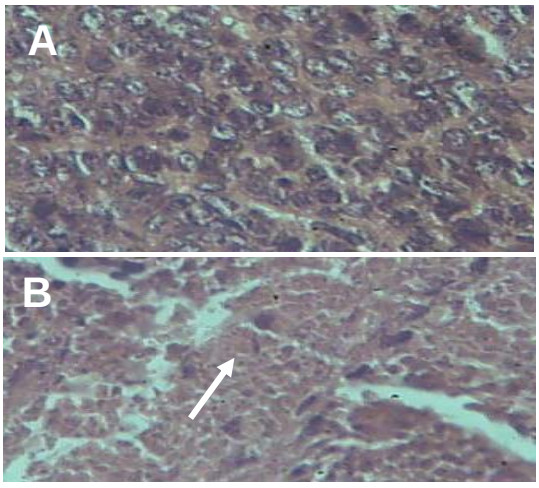

Figure 9 Tumor histopathology; ***A***, Group 2 (cancer, untreated), showing sheet of pleomorphic hyperchromatic cells; ***B***, arrow indicates necrosis of tumor cells.

Table 5 Key histopathological findings.

Group	No. of animals	Liver	Spleen	Kidney	Tumor
1	5	Kupffer cell proliferation; congested central vein; lymphocyte aggregation around central vein *(Fig. 5 B)*	White pulp hyperplasia with amyloid-like substance deposition in red pulp *(Fig. 6 B)*	Congested blood vessels *(Fig. 7 B)*	Necrosis of tumor cells *(Fig. 9 A)*
2	5	Presence of tumor-associated macrophages (TAM) *(Fig. 5 A)* ***"Metastatic tumor"***	Congestion of red pulp and tumor-associated macrophage present in congested blood vessel *(Fig. 6 C)* ***"Metastatic tumor"***	Heavy growth of tumor cells around glomeruli (disapperance of tissue features) *(Fig. 7 A)* ***"Metastatic tumor"***	Sheet of pleomorphic hyperchromatic cells *(Fig. 9 B)*
3	5	Presence of kupffer cells and monocyte aggregation *(Fig. 5 C)*	Hyperatrophy of medial layer of central artery with hyperplasia of periarterial sheet *(Fig. 6 A)*	No changes	–

subsequent suppression of angiogenesis.[7,25] Some flavonoids with a 5-OH exhibited lower cytotoxicity than their non-hydroxylated counterparts; therefore, results indicated that 3,6 dihydroxyflavone showed the most potent cytotoxic effect on cancer cells (breast cancer, prostate cancer, and colorectal carcinoma cells). It has been reported that flavonoids in brownish scale of onion effect growth inhibition of tumor by reducing the fluidity of tumor cell membranes,[26] while quercetin inhibited the expression of specific oncogenes and genes controlling cell cycle.[27] Moon and his colleagues[28] revealed that cancer protection by flavonoids may include alteration in detoxification enzyme (phase I and phase II) which are known for their role in the metabolism of foreign compounds, and many carcinogens are metabolized by these enzymes system to biologically inactive metabolites. One possible mechanism suggested that apigenin may exhibit its cytotoxic effect on tumor cell by its antioxidant and free radical scavenging activities.[29] As all flavonoids compounds, apigenin may play a part in indirect inhibition of NF-KB factor, in addition to inhibition of its binding to DNA strand and transcription[30] and or activation of P_{38} MAPK.[31]

Malondialdehyde (MDA) and glutathione (GSH)

Increment in tissues MDA concentration and formation of MDA as a by-product of lipid peroxidation (LPO) has been considered a simple and useful diagnostic tool for the measurement of LPO.[32]

However, the result of heart, liver, kidney and tumor mass of (AMN3) homogenates of mice after 24 days, of apigenin treatment showed a significant decline (Table 2) in malondialdehyde concentration as compared to the control and other treated groups. These results confirmed the antioxidant role of apigenin, and their free radical scavenging properties.[33] Therefore, apigenin administration was accompanied with significant increment in antioxidant capability and peroxidation inhibition which was reflected by a significant decrease in MDA concentration. Higher MDA and lower GSH concentration confirmed the oxidizing role of cancer in lipid peroxidation,[34,35] reflecting glutathione turnover for preventing oxidative damage in the mice. Similar reports of lowered glutathione concentration in cancers have been reported earlier.[33,36] In addition, several studies[35,37,38] reported similar findings in patients with malignant breast tumor, and with colorectal cancer tissue. While glutathione detoxification system is one of the defense system against free radicals and carcinogen, lower levels of glutathione may favor an over production of free radicals and lipid peroxides, which in turn may induce damage to the cell membrane and cellular molecules (i.e. DNA, RNA) leading to neoplasia. Similar lower erythrocytes glutathione levels in cervical cancer patient were reported by.[39] They have hypothesized that there may be an impairment of the glutathione scavenger system due to the carcinogenic process.[40]

Agarose Gel Electrophoresis

The changes in plasma protein concentration in a cancer-bearing state may not be a specific tumor-induced stimulus, but rather a generalized response to an inflammatory state.[41,42] Mice with AMN3 were characterized by a considerable alteration in the serum protein as compared to control or apigenin-administered group. However, in this study serum albumin decreased in cancer-bearing mice similar to an acute inflammatory stress,[41] dehydration and malnutrition,[43] and albumin decrement coincided with α1-, α2-, β- and γ-globulin decrement as a result of tissue damage and autoimmune disorders. In addition, albumin decrement may be attributed to the high level of catabolism and, as a consequence of cancer, there was a possibility of free radical increment which play a major role in LDL oxidation, accompanied with elevation of β-globulin LDL-vehicle.[42]

The higher permeability of cell membrane in cancer-bearing mice may be considered another factor for albumin decrement. Elevation in α1-globulin in cancer group may be due to increased

trypsin enzyme in the lung that breaks down protein[42] as a result of inflammation, and tissue damage.

Concerning α1- and α2-globulin concentration in apigenin-treated group, the documented result revealed the effective role of apigenin in the improvement of the level of those fractions through elevation of lymphocyte stimulation index. Therefore apigenin has a certain immune co-stimulation effect.[45]

The radical scavenging capability of the apigenin may reduce LDL-C-oxidation and β-globulin fractions required for the transportation of LDL-C.[43] A considerable elevation in γ-globulin was observed in cancer bearing mice treated with apigenin, and this may be attributed to immune system stimulation. Liver and spleen recorded prevascular coughing of lymphocytes which indicated immunity stimulation.[46] Low values of gamma globulines was also recorded in leukemia, cancer, starvation and sever dietary deficiency.[43,47]

Histopathological study

*Liver***.** Lesions occurred as a result of oxidative damage by cancer, while apigenin treated mice showed an increment in kupffer cells and lymphocyte cells aggregation around central veins and there was cancer cells disappearance (Figure 5B) as compared with untreated group. Such results confirmed the hepatoprotective role of apigenin[44] while the liver of normal mice treated with apigenin showed kupffer cells and lymphocytes aggregation of and macrophages in parenchyma and around central vein (Figure 5C) and this may be attributed to the anti-inflammatory action of apigenin.[48]

*Spleen***.** Deposition of amyloidosis (diffuse) in spleen may occur because of high level of immuno-globulins and proteinaceous substances in the blood serum. Amyloid precipitation is reversible pathological changes and fetal in human and animals.[49]

These results explained that apigenin may stimulate tumor necrotic factor (TNF), a group of cytokines with important functions in immunity, inflammation, differentiation, control of cell proliferation and apoptosis. These cytokines induce cell death through sequential recruitment by death receptors and rapid activation of cascade of caspases.[50]

Conflict of interest

None to declare.

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