Where do we stand with fermented wheat grain extract for cancer treatment: lights and shadow?

Preprint · August 2021

DOI: 10.13140/RG.2.2.25241.54887

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Where do we stand with fermented wheat grain extract for cancer treatment: lights and shadow?

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ABSTRACT

Natural products are an important source of new drugs in medicine in general and in oncology in particular. They may require synthetic modifications to improve their results or improve their therapeutic window. But the origin still remains to be the natural predecessor. Many of these drugs swiftly entered medical practice. Others remained in a controversial limbo. This is the case of the fermented wheat grain extracts.
Fermented wheat grain extract (FWGE) was found to have anti-tumoral effects. A large part of the research on these effects was carried out by the same investigators that patented the extract or by close associates. This fact led to some doubts and no little criticism by lay publications. Its classification as a nutritional supplement and its over the counter availability cooperated to a skeptic vision of the extract by the scientific community. Another problem is that being a mixture of compounds, no specific chemical has been identified beyond certain doubts regarding the anti-tumoral effects. 2-methoxy benzoquinone and 2, 6-dimethoxy benzoquinone are probably two of the chemicals behind the pharmacological effects. However, impaired glucose utilization seem to be produced by some other, as yet unidentified fraction of FWGE. Protein extracts from FWGE have shown independent and powerful antitumoral effects.
In spite of the scientific doubts developed behind this compound, FWGE has anti-tumoral effects that were confirmed by many independent researchers. The unfortunate early commercial approach without sound clinical trials should not preclude serious studies of a potentially useful drug, that has some metabolic and immunologic effects not achieved by other chemotherapeutic pharmaceuticals with no toxicity.
This review intends to analyze all the evidence coming from independent, reliable and not biased sources.

INTRODUCTION

Since history of Medicine started to be recorded, natural products have been an important source of useful drugs. Before the nineteenth century they were the only origin of therapeutics. Advent of scientific modern chemistry introduced new synthetic molecules and also modified many old ones improving their performance. However, nature is an inexhaustible fountain of new products even nowadays.
Fermented wheat grain extract, an easily obtainable anti-tumoral compound, is a natural product that after more than 30 years of research has not been accepted as a mainstream cancer drug. As far as it could go, was to be considered a nutritional supplement for cancer patients.
In 2011, a Hungarian web newspaper Valasz.hu published an article by **Élő Anita** under the title “The big business of cancer” (http://valasz.hu/itthon/a-nagy-rakbiznisz-43804/)
The main heading said: “*Hungarian researchers found the antidote to cancer - they immediately discovered a dozen of them. Although authorities are punishing miracle cure*

manufacturers one after the other, these fines have no deterrent effect on millions of items on the multibillion-dollar market".
The article was targeting a product called Avemar® which was patented by Rita Tömösközi-Farkas, Károly Lapis, Erzsébet Rásó, Béla Szende, and Mate Hidvegi after a research that started in the 1980s[1]. All five were seasoned researchers.
According to the article by **Élő Anita**, Avemar producers were fined with 12.975 forint (less than 500 US dollars at the time) for misleading advertisement of the product as a cancer drug.
Avemar is the brand name of the extract of the industrial fermentation of wheat grain (FWGE). Most of the research on FWGE's anti-tumoral activity was developed by Hungarian researchers mainly in relation with the patent holders. Hungary's National Institute of Food Safety and Nutrition approved Avemar as a medical dietary supplement to be used in cancer patients…but not as a therapeutic drug.
Wheat germ is fermented by *Saccharomyces cerevisiae*, then dried and granulated. Therefore, it is not a substance but a mixture of different compounds. Supposedly, two quinones found in the extract are responsible for the biological effects: 2-methoxy benzoquinone and 2, 6-dimethoxy benzoquinone.
Avemar is the aqueous extract of fermented wheat germ, dried with maltodextrin, and standardized to contain approximately 200 μg/g of 2,6-dimethoxy-p-benzoquinone. Classified as a nutritional supplement it showed no toxicity at very high doses, lacking genotoxicity or mutagenicity with doses 25-fold higher than those usually employed[2].
Many publications have backed the evidence of FWGE`s significant anti-tumoral effects. However, a big part of these publications came from researchers who were patent holders or had some commercial interests in the product.
It is the purpose of this paper to explore the bibliography on FWGE excluding the research performed by any possibly biased investigator or with commercial interests, and/or with relations to the patent holders and/or Hungarian government, in order to establish the real effects of the extract on an independent basis. Hereby we shall try to answer two questions:

Is FWGE a scientifically backed resource for cancer treatment?
Is there enough independent unbiased evidence showing its anti-tumoral effects?

MATERIAL AND METHODS

A search was made through PUBMED and Scholar Google with the words Avemar, Fermented wheat germ extract, FWGE. Reviews were excluded. Articles by patent holders were also excluded. Research carried out in Hungarian hospitals or laboratories were excluded.
48 articles related with AVEMAR were found in PUBMED for further analysis.

FINDINGS (in a chronological order)

Anti-proliferative and anti-glycolytic effects

► (1999) Guifa et al.[3] from the Shandong Medical University, China, using a flavonoid extract of wheat germ found that it inhibited a breast cancer cell line growth in a dose dependent manner.

► (2002) Comin-Anduix et al.[4] from the Department of Biochemistry and Molecular Biology, CeRQT-PCB at Barcelona Scientific Park, University of Barcelona, Spain, found that a concentration of 0.2 mg/ml (200 µg/ml) of Avemar had cytotoxic effects on Jurkat cells in a concentration dependent manner. Importantly, they found that this product decreased the glycolytic flux and the pentose phosphate pathway. Apoptotic effect took place through proteolysis of poly(ADP-ribose) showing that it was caspases-dependent.

Anti-proliferative effects on human colon cancer cells and promyelocytic leukemia cells.

► (2005) Illmer et al.[5], from the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, General Hospital of Vienna, Austria, found that Avemar had the ability to inhibit proliferation of HT-29 colon carcinoma cells inducing apoptosis and necrosis at an average concentration of 120 µg/ml. These effects were increased by adding vitamin C. Avemar showed inhibitory effects on ribonucleotide reductase, Cox 1 and Cox 2 enzymes.
The same research team confirmed the apoptotic and ribonucleotide reductase inhibitory effects in human leukemia promyelocytic cells[6] (2007) and reversal of resistance to 5-FdUrd/Ara-C in H9 human lymphoma cell (2009)[7].However, we exclude this last publication from our analysis because among the twelve authors, one is a patent holder.

Cytotoxicity in human gastric carcinoma cells

► (2005) In a study performed in Korea, FWGE induced apoptosis in human gastric carcinoma cell lines (SNU-1, SNU-5, SNU-16, SNU-620, MKN-45) in a dose dependent manner and with IC50 ranging between 370 and 620µg/ml according to the different lines[8].

Improved survival in high risk skin melanoma patients

► (2008) Demidov et al.[9] from the N. N. Blokhin Cancer Research Center and the Russian Academy of Medical Sciences, Moscow, Russian Federation, carried out a randomized, phase II clinical trial, comparing the results of melanoma patients treated with dacarbazine (DTIC) alone with patients receiving dacarbazine plus a 1-year administration of FWGE. Results were very significant : mean PFS: 55.8 months with FWGE versus 29.9 months without. Mean OS: 66.2 months with FWGE versus 44.7 months without.

Apoptotic effect in general

► (2011) Mueller et al.[10] from the University of Halle, Saale, Germany tested FWGE against 32 different tumor cell lines. FWGE showed significant anti-tumor activity in all of them inducing apoptosis at different concentrations. Neuroblastoma cell lines were particularly susceptible where an average concentration of 42 µg/ml was enough to produce apoptosis of 50% of cells. Additive to synergistic effects were found with 5FU, irinotecan and oxaliplatin. In a previous poster presentation by the same group[11], they found that FWGE was particularly effective in apoptosis induction in glioblastoma, testicular and ovarian cancer. In colon cancer cell lines FWGE showed synergistic effects with 5-FU.

Anti-proliferative effects on human ovarian cancer cell lines and potentiation of cisplatin

► (2012) Judson et al.[12] (from H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA) used Avemar against different lines of ovarian cancer cells. A median concentration of 244 µg/ml induced apoptosis and increased sensibility to cisplatin.

Cytotoxic effects in hepatocarcinoma and synergy with cisplatin and 5FU.

► (2013) Thai et al.[13] from the Taipei Medical University Hospital found that FWGE exhibited suppressed growth of HepG2, Hep3B, and HepJ5, hepatocarcinoma cells with concentrations that ranged between 370 and 1500 µg/ml and enhanced cytotoxicity of chemotherapeutic drugs such as cisplatin and 5FU in all different cell lines.

Cytotoxic effects on ovarian cancer cells

► (2015) Wang et al.[14] from Taipei Medical University Hospital, Taiwan, found that FWSE had the ability to inhibit ovarian cancer cells (SKOV-3 and ES-2), inducing apoptosis with a concentration of 643.76 µg/ml and 246.11 µg/ml, respectively. It added cytotoxic effects to cisplatin and docetaxel.

Tumor growth inhibition in xerotransplanted mice

► (2015) Zhang et al.[15] from Jiangsu University, China, treated nude mice with xerotransplanted HT-29 cells (human colon adenocarcinoma) with lacto-fermented wheat germ extract finding that tumor size and weight were substantially decreased compared with untreated mice.

Anti-proliferative and anti-metastatic effects on oral cancer cells

► (2016) Yang et al.[16] from the China Medical University Hospital , Taichung , Taiwan found that AVEMAR treatment of oral cancer cells SCC-4 at a concentration of 0.2-1.6 mg/ml (200-1,600 µg/ml) inhibited cell viability and had the following effects: induced cell apoptosis, inhibited migration and invasion of metastatic cells and suppressed MMP2 and urokinase plasminogen activator (uPA) expression, but not MMP-1 or MMP-9.

Different actions of benzoquinones and FWGE

► (2016) Otto et al.[17], from the University Hospital of Würzburg, Germany compared the effects of FWGE and the dimethylbenzoquinone component finding that both induced oxidative stress that promoted cytotoxicity. However there was an FWGE effect that benzoquinone lacked: growth delay associated with impaired glucose utilization. Therefore, FWGE contains some other fraction, not identified as yet that modified cancer cell metabolism by restricting the glycolytic flux leading to autophagia.

In vivo inhibition of HT-29 human colon cancer cell growth

► (2016) Jiayan et al.[18] from Jiangsu University, China, showed that FWGE inhibited colon tumor cells growth when xerotransplanted in mice.

Antiangiogenic effects

► (2017) In a research performed at Akdeniz University, in Antalya, Turkey[19] Avemar was tested in gastric tubular adenocarcinoma, PC3 prostate carcinoma, HeLa and lung adenocarcinoma cells. AVEMAR concentrations between 400 and 3,200 µg/ml were used. The treatment resulted in a significant VEGF and Cox-2 mRNA decrease in gastric adenocarcinoma and HeLa cells compared with those untreated, in a concentration dependent manner. The other cell lines also showed decreased VEGF and Cox2 expression but less significant than gastric or HeLa cells. We found no other independent research report on Avemar's antiangiogenic effects.

Anti-inflammatory effects

► (2017) There is more than one procedure to release benzoquinones from wheat germ. Fermentation is the generally used method. Jeong et al.[20] from Kyung Hee University, Korea, instead of fermenting wheat they extracted benzoquinones by treating the germs with citric acid. Then they tested its anti-inflammatory properties and found that it decreased/inhibited the production of pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-6, and IL-12 and reduced COX2 generation. It also reduced NF-kB activity and increased IL-10.

Cytotoxic and pro-apoptotic effects on ovarian cancer cells, reduction of ROS and NF-kB activity.

► (2017) Koh et al.[21], from the Korea Institute of Toxicology, studied FWGE effects on ovarian cancer cell lines OVCAR-3 and SK-OV-3. Significant anti-proliferative action and apoptosis were found. They also observed an important reduction of experimentally induced ROS production and NF-kB activity and increased activity of NK cells against OVCAR.

FWGE antitumoral effects are not only bemzoquinone-dependent

► (2014) Abuhay et al.[22] purified proteins from FWGE and found them more potent against cancer than FWGE, being cytotoxic for B lymphocyte cancer cells and a potent immunomodulator: increased CD4+ T cells, NK-T cells, and activated monocytes.

► (2018) Barisone et al.[23] from the University of California showed that a purified protein fraction of FWGE had potent lymphomacidal abilities in vitro and in vivo. The mechanisms involved were increased immune activity by NK cell killing activity, and increased IFNγ production..

2-6-dimethoxibenzoquinone (2-6-DMBQ) inhibits the mTOR/Akt pathway

► (2021) Xie et al.[24] found that 2-6-dimethoxy-benzoquinone had anti-tumoral effects suppressing proliferation and migration through the mTOR/AKT pathway inhibition and p38 MAPK signaling in non small cell lung cancer cells. Additionally, it inhibited also the expression and phosphorylation of cyclin B1 and CDC2. Migration decrease/inhibition was a consequence of E cadherin increased expression.

► (2020) Xu et al,[25] from Zhengzhou University, China, also found that mTOR inhibition produced by 2-6-DMBQ induced gastric cancer cells apoptosis and reduced proliferation. Growth inhibition by 2-6-DMBQ was mTOR inhibition-dependent.

The proof of concept

Rizzello et al.[26] from the University of Bari, Italy, fermented wheat germ with different Lactobacillus bacteria. After 24 hour of incubation 2-methoxy benzoquinone, and 2,6-dimethoxybenzoquinone generation was completed. These are the same chemicals that are supposedly behind the anti-tumoral activity of Avemar. (Figure 1). Next, they tested the biological activity of raw wheat germ with no fermentation and the fermented sourdough with various tumor cell lines such as colon and ovarian carcinoma. Non-fermented wheat showed no effect, while the fermented significantly affected cellular proliferation with IC_{50} values ranging from 100 to 556 μg/ml. The fermented wheat sourdough had 4 to 6 fold higher content of benzoquinones when compared with non fermented. Interestingly, the antitumoral activity of sourdough was similar to what was found with Avemar.

However, benzoquinones, are not the only antitumoral components of FWGE. There is a long list of polypeptides included in it, that may have anti-cancer effects (these polypeptides are termed FWGP). A group of US patents granted to the University of California (US18008909P, US20120121612A1, US9480725B2) gives a long list of low molecular weight polypeptides contained in fermented wheat grain supposedly having anti-cancer properties. Table 1 of this patent lists more than 70 different polypeptides with molecular weight ranging between 5 and 100 kD and they are all supposedly "anti-cancer". No specific prove is given on this "anti-cancer" properties for each protein. The administration route should preferentially be parenteral or, if oral route is used, the polypeptides should be protected against gastrointestinal destruction. The different cytotoxicity of the polypeptides fractions separated with a Cephadex column were tested using Raju lymphoma (NHL) cell line. A comparative cytotoxicity study at cellular level and with xenografted mice between FWGE and FWGP showed that both had anti-tumor effects: *"The data in Experiments 16 and 17 verified that FWGE and FWGP had significant in vivo efficacy in a human lung cancer model"* and "*both FWGE and FWGP were potent complement activators"*.

We believe that the pharmaceutical oral form of FWGE used for Avastin cannot be expected to develop effects such as shown for FWGP mainly because of gastrointestinal degradation of the polypeptides. However, these patents are a fully independent proof of concept that:

1) FWGE has cytotoxic effects on different tumor cells.
2) FWGE has other cytotoxic components besides benzoquinones.
3) FWGE improves immunological defenses through increased complement and increased activity against tumors through immune mechanisms.

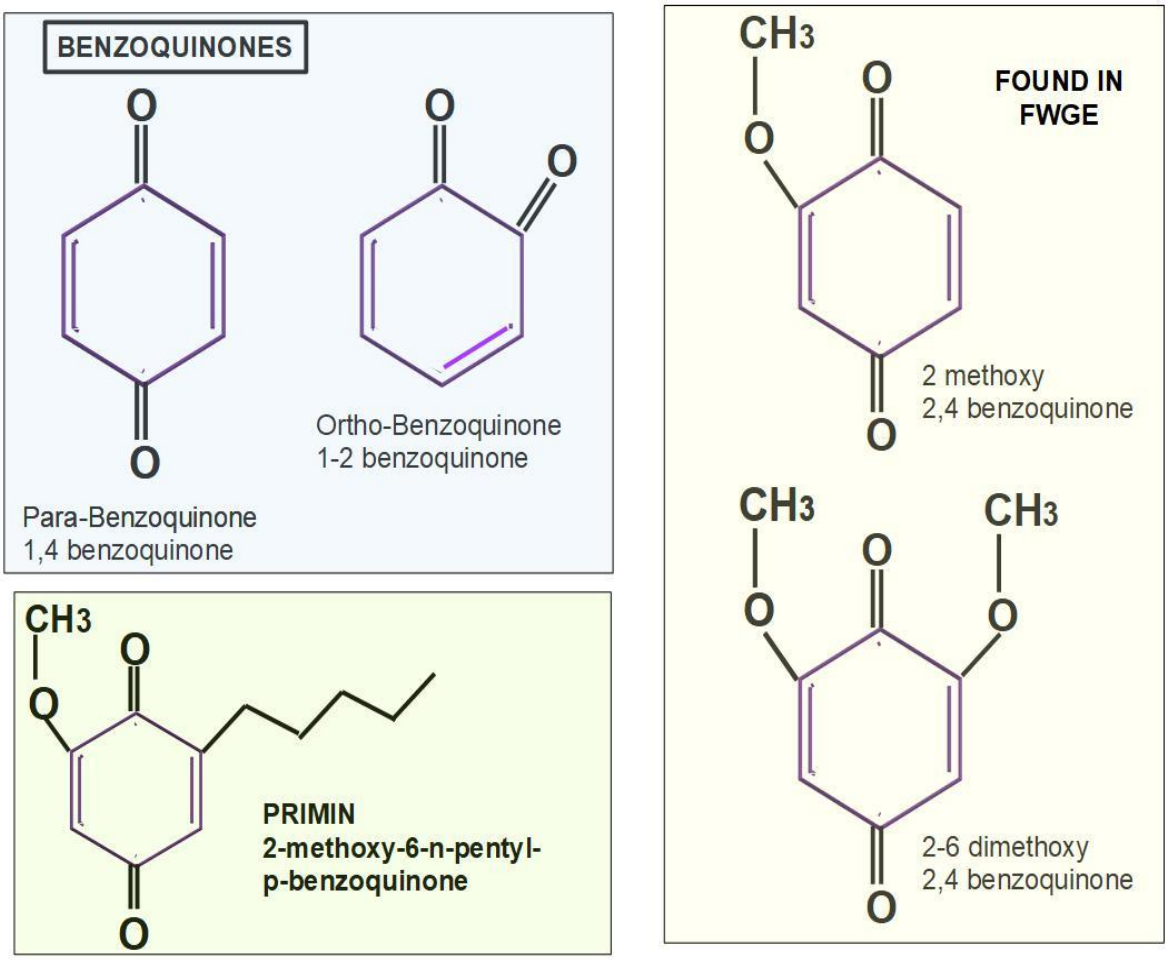

Figure 1.- Chemical structure of the two main components of FWGE (right panel). Data downloaded from https://hmdb.ca/metabolites/HMDB0032576. Left lower panel shows the chemical structure of primin, an interesting anti-tumoral benzoquinone. Upper left panel shows the structure of 1,4 para-benzoquinone from which some anti-tumoral derivatives have been found in nature or synthesized. Isolation of methoxy and 2-6 dimethoxybenzoquinone in wheat germ was accomplished by Cosgrove et al. in 1952[27], [28].

Interestingly, Primin,a natural benzoquinone obtained from Primula obconica (Primulaceae) that has a pentyl chain is an anti-tumoral compound[29].
Oximes, derived from 1,4 para-benzoquinone also have anti-tumoral effects. For example 2-methyl-1,4 benzoquinone oxime tosylate derivatives have shown anti-proliferative effects against different tumor cells at very low concentrations[30]. Figure 2.

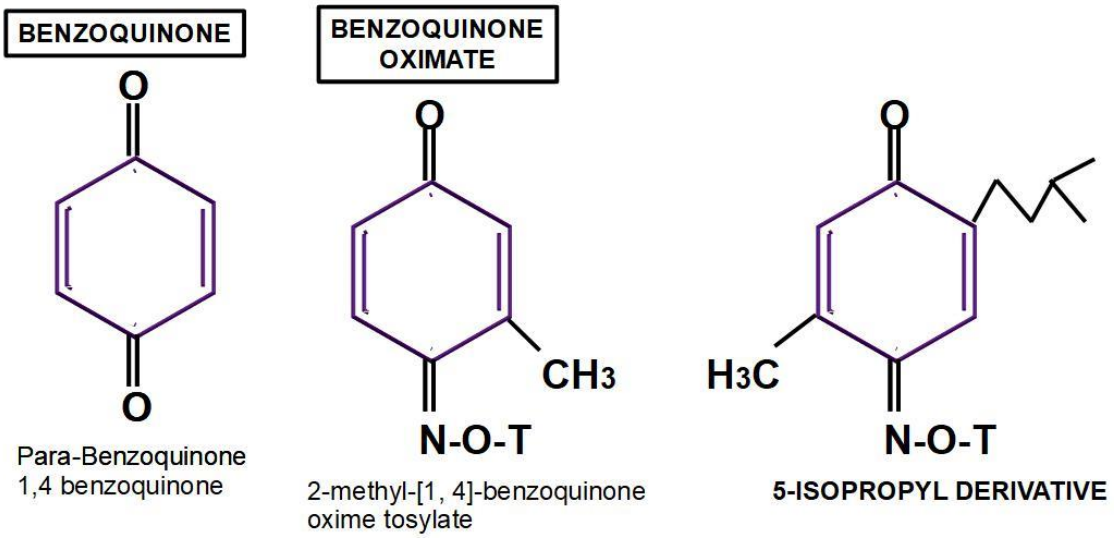

Figure 2.- Benzoquinone oximate derivatives with anti-tumoral effects. The 5-isopropyl derivative is effective against proliferation of HL-60 (promyelocytic leukemia), MCF-7 (breast adenocarcinoma) and NCI-H292 (lung carcinoma). cells with concentrations around 0.3 , 3.6 , and 1.6 µg/ml respectively. Oximate derivatives were synthesized through acidification of phenol with HCL and adding sodium nitrite. Can this happen naturally with benzoquinones in the acidic stomach? We do not know. However, there is a possibility that low amounts of oximates may be formed in the acidic gastric environment that would further explain FWSE anti-tumoral effects. This issue deserves further research. The isopropyl chain in oximates and the pentyl chain in primin seem to be related with enhanced anti-tumoral effects.

Another benzoquinone derivative that was found to exert anti-cancer effects is Evelynin isolated from *Tacca chantrieri* roots showing cytotoxicity against "MDA-MB-435 melanoma, MDA-MB-231 breast, PC-3 prostate, and HeLa cervical carcinoma cells with IC_{50} values of 4.1, 3.9, 4.7, and 6.3 µM, respectively"[31]. (Figure 3)

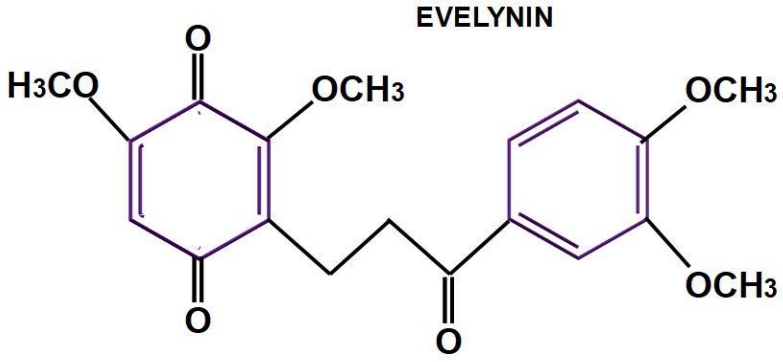

Figure 3.- Evelynin is a benzoquinone with anti-tumor effects.

Evelynin, however, seems to act through a different mechanism than the other benzoquinones: they stabilize microtubules[32], [33], [34].

CLINICAL TRIALS

The clinical trial for melanoma by Demidov et al., already mentioned is probably one of the most important independent trials, where FWGE associated to dacarbazine almost doubled the progression free survival period.

A recent study of FWGE for castration resistant prostate cancer showed some minor benefits, being the main finding a prolongation of the PSA doubling time in 65% of 36 patients. The study was performed in Sheba Medical Center, Israel, however, we excluded this report because one of the patent holders is also a coauthor[35].

A multicentric study carried out in Genova, Italy in patients with head and neck cancer, showed that FWGE improved quality of life but there is no mention of any other clinical outcome[36]. Almost all the clinical experience with FWGE comes from non-independent sources, thus independent clinical trials are in need.

DISCUSSION

Dr. Albert Szent-Gyorgyi, 1937 Nobel Laureate for his work on vitamin C, was the first to study the immune modulating properties of wheat plant. He also identified benzoquinones in wheat germ as the possible source of antitumoral effects[37], [38], [39]. Szent-Gyorgyi showed that 2-6-DMBQ was cytotoxic for Ehrlich ascitis tumor cells in vivo and that this cytotoxicity was increased with ascorbic acid[40].

These studies took place in a very late phase of his life and he never concluded the research. However, the idea of obtaining benzoquinones from wheat germ survived and was swiftly taken up by a Hungarian biochemist, Mati Hidvegi who was close to Szent-Gyorgyi and completed the investigation. Since then, he published over 45 articles regarding Avemar and also he is one of the patent holders.

The process to obtain benzoquinones from wheat germ is quite simple (Figure 4), and can be carried out by fermentation or treating the wheat germ with citric acid.

An important issue is that conditions under which fermentation is performed can significantly modify the amount of benzoquinone obtained[41].

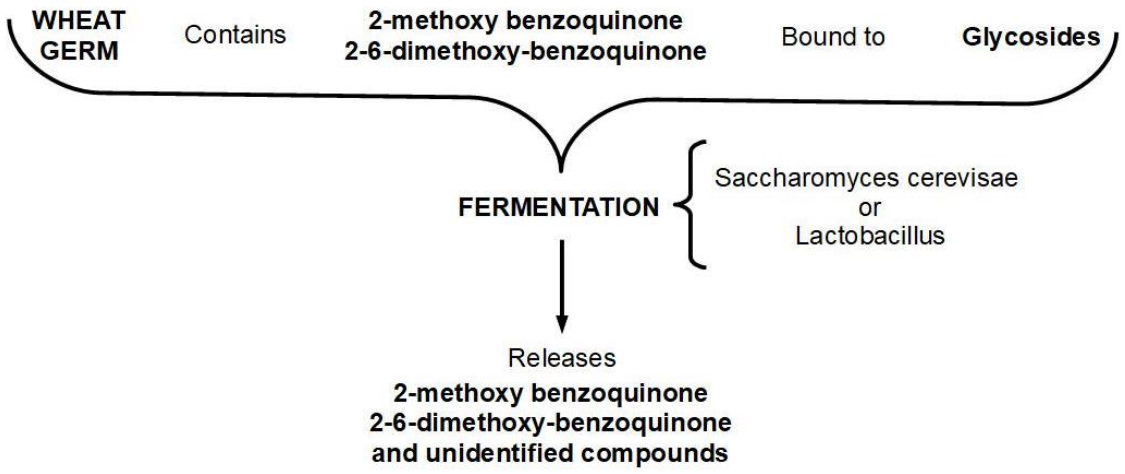

Figure 4.- Releasing glycosylated benzoquinone molecules from wheat germ through fermentation. Composition of wheat (*Triticum aestivum)*: 80% endosperm, 15% bran and 5% germ[42].

Interestingly, DMBQ in normal muscle increases the mTOR/AKT signaling and protein synthesis. It also stimulates mitochondrial respiration and the oxidative enzymatic activity[43]. In tumors, it seems to stimulate mitochondrial respiration but inhibits mTOR/AKT signaling[44], [45]. Does this mean that its activity is context or tissue dependent?
We are unable to answer this question.
2-6-DMBQ was found to inhibit DNA synthesis[46], and histamine release from mastocytes[47]. Morgan et al.[48] found that the cytotoxic effects of 2-6-DMBQ are mediated by the immune system, because when they tested it in immunosuppressed mice cytotoxicity was absent.

In spite of all the lay and scientific criticism harvested by FWGEs, there is strong independent evidence showing that these non-toxic extracts have clear anti-tumoral effects. The mechanisms involved in these effects are:

a) Reduction of the increased glycolytic flux usually found in most highly proliferative tumors.
b) Inhibition or down-regulation of the pentose phosphate pathway through decreased G6PDH and transketolase activities that are key enzymes involved in glucose conversion into the five-carbon nucleotide precursor ribose pool. Stable isotope studies indicate that Avemar is a powerful inhibitor of de novo nucleic acid synthesis. On the contrary, Avemar has no toxic biological effects on normal cells in the doses that affect tumors.
c) Inhibition of the mTOR/AKT pathway.

There is no experimental evidence, but we think that the glycolytic flow is slowed down due to accumulation of unprocessed substrates at the pentose phosphate pathway[49], [50], [51], [52] leading to lactate overproduction which surpasses monocarboxylate transporters ability to extrude lactate, thus creating intracellular lactic acidosis which unleashes acidic stress. Finally, this leads to autophagy or apoptosis. If this hypothesis is correct, adding metformin would further increase intracellular lactic acidosis, accelerating apoptosis.

The concentration issue
According to Comin-Anduix et al., the effective oral dose of Avemar that inhibits tumor metastasis formation is 9.0 g/day, which is equivalent to an estimated plasma concentration of 0.5 and 1 mg/ml in an average (70 kg) weight patient. However, this data is not supported by evidence establishing the 9 g/day dose as achieving the concentration in which it was used on malignant cells. Unfortunately, we found no publication establishing the mean concentration of Avemar in plasma, serum or at tumor level after an oral administration of 9 gm/daily. No minor issue is the fact that Avemar is a mixture of compounds, therefore which compound should be measured in blood or at the tumor level to know the precise drug availability?
Probably, 2-6-DMBQ concentration level would be an adequate proxy.
Therefore, an important question remains unanswered: FWGE achieves concentrations in the order of nanograms or micrograms? In the first case, no clinical results are expectable because all the reported experiments were carried out at concentrations above 100 μg/ml.

The chemicals involved in the antitumoral effects are mainly, but not solely benzoquinones. There is also independent evidence that benzoquinones and closely related drugs such as oximate benzoquinones and evelynin exert tumor inhibitory effects. This chemical group deserves deeper research regarding that it is far from being fully explored.

The protein fraction

Evidence indicates that there are benzoquinone-independent anti-tumoral effects involved in FWGE activity. The more than 50 different polypeptides that had been identified in the extract showed clear anti-tumoral activity. However, there is another unsolved issue: how many of these polypeptides remain unmodified in the gastrointestinal tract with oral administration?

Conclusions:

1) Independent evidence confirms Avemar's anti-tumoral effects.
2) Independent evidence confirms benzoquinone's anti-cancer effects.
3) Independent evidence confirms that polypeptidic fractions of FWGE can enhance immunologic anti-cancer effects and even exert apoptotic activity.
4) Further research is warranted on synthetic modifications of benzoquinones as potential anti-cancer drugs.
5) Serum and tumor concentrations achievable after oral administration of Avemar or benzoquinones should be determined.
6) Independent clinical trials are still necessary to establish the real place of the compound in the therapeutic armamentarium.

Conflicts of interests: Tomas Koltai is a Hungarian citizen that holds no relationship with any of the patent owners and/or the Hungarian government and has been living outside Hungary since 1950. Tomas Koltai does not have or had, commercial or any other ties with Avemar producers, distributors or sellers.
No financial assistance or subsidy or any kind of payment has been received for the present research.
There are no human or animal experiments reported in this paper.

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