ankaferd PROTEOMIX®
Aromatic Plant Extract

Anti-Neoplastic Effect

For Healthcare Professionals Only.

Ankaferd Proteomix

Ankaferd Proteomix is a herbal extract that has been used folklorically in traditional Turkish medicine for centuries. Ankaferd Proteomix is obtained from a standardized blend of Thyme (Thymus vulgaris), Licorice (Glycyrrhiza glabra), Grape Vine (Vitis vinifera), Galangal (Alpinia officinarum), and Nettle (Urtica dioica).

Another pharmaceutical form of Ankaferd is available as a hemostatic agent under the trade name Ankaferd BloodStopper®, licensed by the Ministry of Health of the Republic of Türkiye (the first and only proprietary pharmaceutical molecule developed in Türkiye).

Safety and Efficacy Data:

Based on scientific research, Ankaferd is considered safe in terms of biochemical toxicity,[1] mucosal toxicity,[1] hematotoxicity,[1] hepatotoxicity,[1,2] nephrotoxicity, and neurotoxicity; it carries a low risk of drug interactions; it reduces oxidative stress;[2,3] it is hypoallergenic;, pleiotropic effects, and an anti-infective plant compound.

It has also been demonstrated that the concentrations of antioxidants and other specific molecules contained in Ankaferd are not affected when exposed to synthetic gastric fluid.

Considering the uniquely broad clinical spectrum of Ankaferd, additional toxicity analyses related to its use have been conducted for Heavy Metals, Pesticides, Mycotoxins, GMOs (Genetically Modified Organisms), Dioxins, and Polychlorinated Biphenyls (PCBs), confirming its safety profile.

Immunomodulation refers to any process that modifies the effectiveness or response of the body's immune system. This process may either enhance immune function (**immunostimulation**) or suppress it (**immunosuppression**).

As an effective immunomodulator, Ankaferd Proteomix acts like a conductor of an orchestra, capable of regulating the body's defense system in a delicate and balanced manner. Rather than acting in only one direction, it can assume different roles according to physiological needs.

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Anti-Neoplastic

Ankaferd Proteomix possesses **anti-inflammatory, antimicrobial, antifungal, antioxidant, and antineoplastic** properties. The aim of this review is to summarize the current pharmacobiology of Ankaferd in neoplastic disorders.[1]

Cancer treatment is a challenging clinical condition that can lead to numerous clinical complications of varying severity. The antineoplastic properties of Ankaferd have been scientifically demonstrated in many solid and haematological tumors.[1]

Ankaferd's apoptotic effects—specifically, its ability to trigger programmed cell death (apoptosis)—constitute a key mechanism focused on cancer cells and aimed at their elimination. Current research indicates that Ankaferd can induce apoptosis through the following pathways:

- Increased production of Reactive Oxygen Species (ROS),
- Activation of the mitochondrial pathway
- Activation of the caspase cascade
- Regulation of Bcl-2 family members
- Modulation of death receptor pathways
- Modulation of gene expression

Supportive cancer therapy is critically important in reducing mortality and morbidity in cancer patients. Due to its unique effects on blood cells, endothelium, angiogenesis, cellular regeneration, wound healing, and vascular dynamics, Ankaferd prevents and treats chemotherapy-associated mucositis. These characteristics also make it beneficial in necrotizing enterocolitis. In addition to its supportive and preventive roles in cancer patients, Ankaferd may potentially be used as a chemoembolization agent in intratumoral treatment approaches.[1]

Cancer treatment may result in numerous complications. Conventional cytotoxic chemotherapeutic agents may damage normal tissue cells. Through its chemo preventive, antioxidant, and supportive properties, Ankaferd may help reduce cancer-related complications.[1]

The anti-neoplastic properties of Ankaferd are associated with its unique transcriptomic, proteomic, and metabolomic characteristics. Ankaferd stimulates cellular factors that play important roles in regulating the cell cycle machinery, pro-apoptotic pathways, angiogenesis, signal transduction, and other metabolic pathways. Some of these factors include Nuclear Factor-1 (NF-1), Interferon (IFN)-Stimulated Response Element (ISRE), Protein 2, Androgen Receptor, Cyclic AMP Response Element-Binding Protein (CREB), SMAD2/3, Cyclic AMP Response Element, Stimulating Transcription Factor-1 (ATF-1), Myc-Max, E2F1-5, Peroxisome Proliferator-Activated Receptor (PPAR), E2F6, EGR, Protein 53, and Yin-Yang 1 (YY1).[2]

The primary mechanism underlying the anti-neoplastic effects of Ankaferd is apoptosis. Protease-Activated Receptor 1 (PAR1) is a member of the Protease-Activated Receptor (PAR) family, which belongs to the group of seven-transmembrane G protein-coupled receptors.[3] Ankaferd increases the activation of PAR1, a member of the PAR family. Increased PAR1 activation alters intracellular signalling through coupling with G proteins. PAR1 and EPCR expression in K-562 and Jurkat cells are regulated by Ankaferd. The pro-apoptotic effects of PAR1 and p21 have been demonstrated in Jurkat cells.[1,4]

Ankaferd regulates p21 involvement in pro-apoptosis independently of PAR1 and p53 in leukaemia cells.[1,4]

Furthermore, Ankaferd possesses various pleiotropic properties, including anti-neoplastic and antimicrobial activities as well as tissue-supportive effects. It increases the expression of CREBZF, which leads to activation of the anti-neoplastic protein p53.[1,4]

Moreover, HNF-4α is a component associated with Ankaferd and possesses anti-neoplastic properties.[1,5]

ME-1, which has significant effects on cancer metabolism, is also among the components associated with Ankaferd.[1,6]

Ankaferd increases the levels of the tumor suppressor proteins UCHL1 and RPL5.[1,7]

Ankaferd stimulates transcription factors involved in various biological mechanisms such as infection, cellular growth, and inflammation, including TRE/AP1, E2F6, AP2, AR, CREB, CREATF1, E2F1-5, EGR, ISRE, HNF1, Myc-Max, NF1, NF-κB, p53, PPAR, GATA, SMAD2/3, SP1, and YY1.[8]

Similarly, Ankaferd enhances transcription factors that regulate cellular growth, including AP2, AR, SMAD2/3, CRE-ATF1, CREB, E2F1-5, ISRE, E2F6, EGR, Myc-Max, NF1, NF-κB, TRE/AP1, p53, PPAR, SP1, and YY1.[8]

Ankaferd exhibits anti-neoplastic properties on lymphoid cells.[9]

At higher concentrations (>0.5 µg/mL), Ankaferd demonstrated anti-proliferative effects in chronic lymphocytic leukemia cell lines, whereas at lower concentrations (<0.5 µg/mL), it was found to enhance cellular differentiation.[9]

Anti-proliferative effects of Ankaferd have also been demonstrated in myeloma cell lines.[10]

Through reactive oxygen species (ROS)-generating, genotoxic, cytotoxic, and pro-apoptotic mechanisms, Ankaferd exhibits anti-cancer effects in melanoma cells.[11]

The anti-tumoral properties of Ankaferd on SaOS-2 osteosarcoma cell lines have been demonstrated in previous studies.[12]

Human CaCo-2 colon cancer cells lost their proliferative characteristics following treatment with Ankaferd.[13]

Ankaferd exerts anti-neoplastic effects on bladder cancer cells. A reduction in the viability of bladder cancer cells has been observed following treatment with Ankaferd.[14]

Ankaferd induces necroptosis in breast cancer cell cultures.[15]

HEPG2 hepatocellular carcinoma cells were inhibited following exposure to Ankaferd.[16]

Inflammation, prematurity, and oxidative stress may contribute to the development of necrotizing enterocolitis. Through its anti-inflammatory, antioxidant, and anti-apoptotic properties on intestinal tissue cells, Ankaferd has demonstrated a protective effect against intestinal injury associated with necrotizing enterocolitis.[17]

Furthermore, hepatocytes may benefit from the antioxidant and hepatoprotective properties of Ankaferd, which contains significant amounts of vitamin E, magnesium, vitamin B12, vitamin D, vitamin B9, vitamin A, calcium, and other trace elements.[18,19,20,21,22]

Transarterial chemoembolization (TACE) aims to localize chemotherapeutic agents directly to the tumor region. In selected patients, this treatment is preferred because of its minimal systemic toxicity. Traditionally, TACE involves ethanol and lipiodol embolization to destroy tumors. Although TACE is considered a safe procedure for targeting tumor tissue, various complications may occur. While local complications are generally expected, systemic complications such as tumor lysis syndrome and metabolic disturbances may also develop. Ankaferd has potential for intratumoral therapeutic applications. The anti-neoplastic properties of Ankaferd in myeloma cell lines have been evaluated in BALB/c mice through in vitro and intraperitoneal studies.[23,24,25,26]

Additionally, Ankaferd has been administered as an embolization agent in splenic and renal arteries in experimental animal models undergoing nephrectomy and splenectomy.[27,28,29]

Oral mucositis is a significant chemotherapy-associated complication in cancer patients and affects approximately 40–80% of patients. The role of Ankaferd in the treatment of chemotherapy-induced oral mucositis in patients with hematological malignancies has previously been demonstrated. Ankaferd has also been shown to be effective in the treatment of oral mucositis associated with anticancer therapies in pediatric cancer patients. [30] Furthermore, beneficial effects have been reported in adults with chemotherapy-induced oral mucositis. [31]

Ankaferd may reduce epithelial dysplasia. It has been demonstrated that Ankaferd reduces oral epithelial dysplasia associated with 7,12-dimethylbenz[a]anthracene.[32]

Ankaferd may prevent oxidative DNA damage. The effects of Ankaferd on superoxide dismutase, 8-hydroxy-2'-deoxyguanosine, and myeloperoxidase levels have been demonstrated in rabbits with pulmonary parenchymal injury.[33]

The protective effect of Ankaferd against oxidative DNA damage has been confirmed experimentally. In addition, Ankaferd possesses antioxidant and antimutagenic properties. These effects have been evaluated using β-carotene–linoleic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. Ames Salmonella/microsome mutagenicity tests using *Salmonella typhimurium* TA98 and TA100 strains were performed to evaluate the antimutagenic activity of Ankaferd. The antioxidant and antimutagenic properties of Ankaferd have been demonstrated in these studies.[34]

Cancer Type	Study Summary
Lymphoid Neoplastic Cells	Proliferation of chronic lymphocytic leukemia cells treated with Ankaferd was inhibited. Transformation of lymphocytic leukemia cells into aggressive blastic lymphoid forms was prevented by Ankaferd.[9]
Multiple Myeloma Cells	Ankaferd demonstrated anti-neoplastic effects on myeloma cells identified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay in BALB/c mice.[10]
Melanoma Cells	Following Ankaferd treatment, reduced viability was observed in SK-MEL-10 (CVCL_6020), SK-MEL-9 (CVCL_U934), A2058 (ATCC® CRL-1147™), and MeWo (ATCC HTB-65™) melanoma cell lines.[35]
Melanoma Cells	Ankaferd induced DNA damage, apoptosis, and increased ROS (Reactive Oxygen Species) levels in melanoma cells.[11]
Osteosarcoma Cells	Following Ankaferd administration, dose-dependent reductions in proliferation and survival were observed in Saos-2 cells.[12]
Colon Cancer Cells	Ankaferd exerted an inhibitory effect on the proliferation of CaCo-2 cells. Following exposure to Ankaferd, these cells lost viability.[13]
Colon Cancer Cells	Ankaferd affects glucose, fatty acid, and protein metabolism as well as cell-cycle mechanisms. It was also found to induce critical cancer target and tumor suppressor proteins such as Carboxyl-terminal Hydrolase 1, 60S Ribosomal Protein L5, Tumor Protein D52-like 2, Karyopherin Alpha 2, and Protein Deglycase DJ-1.[36]
Bladder Cancer Cells	Ankaferd induced apoptosis in bladder cancer cells and reduced cell viability. Necroptosis was observed following treatment.[14]
Breast Cancer Cells	Ankaferd induces apoptosis in breast cancer cells and decreases cell viability. Both necroptosis and apoptosis were identified in breast cancer cell cultures. Cytotoxic effects on breast cancer cells were observed.[15]
Hepatocellular Carcinoma	Ankaferd inhibited the viability of HEPG2 hepatocellular carcinoma cells.[16]

Table: Anti-neoplastic properties of Ankaferd

REFERENCES

1. Malkan UY, Haznedaroğlu İC. **Pharmacobiology of topical Ankaferd hemostat in neoplastic disorders.** *The European Research Journal.* 2023;9(5):1271–1276.
2. Haznedaroglu BZ, Beyazit Y, Walker SL, Haznedaroglu IC. **Pleiotropic cellular, hemostatic, and biological actions of Ankaferd hemostat.** *Critical Reviews in Oncology/Hematology.* 2012;83:21–34.
3. Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. **Proteinase-activated receptors.** *Pharmacological Reviews.* 2001;53:245–282.
4. López-Mateo I, Villaronga MA, Llanos S, Belandia B. **The transcription factor CREBZF is a novel positive regulator of p53.** *Cell Cycle.* 2012;11:3887–3895.
5. Walesky C, Apte U. **Role of hepatocyte nuclear factor 4 alpha (HNF4α) in cell proliferation and cancer.** *Gene Expression.* 2015;16:101–108.
6. Wen D, Liu D, Tang J, Dong L, Liu Y, Tao Z, et al. **Malic enzyme 1 induces epithelial–mesenchymal transition and indicates poor prognosis in hepatocellular carcinoma.** *Tumor Biology.* 2015;36:6211–6221.
7. Ataman MB, Bucak MN, Çoyan K. **Esterified glucomannan improves aflatoxin-induced damage of sperm parameters during liquid storage of ram semen at 5°C.** *Cryobiology.* 2014;68:405–410.
8. Yılmaz E, Güleç Ş, Torun D, Haznedaroğlu İC, Akar N. **The effects of Ankaferd Blood Stopper on transcription factors in HUVEC and the erythrocyte protein profile.** *Turkish Journal of Hematology.* 2011;28:276–285.
9. Akalın İ, Okur FV, Haznedaroğlu İC, Sayınalp N, Aksu S, Büyükasık Y, et al. **Acute in vitro effects of ABS (Ankaferd Hemostat) on lymphoid neoplastic cells (B-CLL and RAJI tumor cell lines).** *International Journal of Hematology and Oncology.* 2014;24:253–259.
10. Avcu F, Guner M, Misirci M, Elci P, Safali M, Goker H, et al. **Evaluation of anti-neoplastic effects of a new hemostatic agent, Ankaferd Blood Stopper, on myeloma cell line and plasmacytoma development in BALB/c mice: Results of the first in vitro and in vivo study.** *Blood.* 2014;124:5728.
11. Kocyigit A, Guler EM, Haznedaroglu IC, Malkan UY. **Ankaferd hemostat induces DNA damage, apoptosis and cytotoxic activity by generating reactive oxygen species in melanoma and normal cell lines.** *International Journal of Clinical and Experimental*

Medicine. 2017;10:2116–2126.
12. Goker H, Cetinkaya D, Kilic E, Haznedaroglu I, Kirazli S, Firat H. **Anti-cancer activity of Ankaferd Blood Stopper on osteosarcoma (SAOS-2) cell lines in vitro.** In: *Ankaferd: Scientific Perspectives and Basic Clinical Data.* Istanbul: Naviga Publications; 2008. p.109.
13. Goker H, Kilic E, Cetinkaya D, Buyukasik Y, Aksu S, et al. **Anticancer activity of Ankaferd on human colon cancer (CACO-2) in vitro.** In: *Ankaferd: Scientific Perspectives and Basic Clinical Data.* Istanbul: Naviga Publications; 2008. p.108.
14. Sarı H, Çelik S, Çağlar F, Aktaş S, Bozkurt O, Yörükoğlu K, et al. **A candidate antineoplastic herbal agent for bladder cancer: Ankaferd Blood Stopper.** *International Journal of Clinical Practice.* 2021;75:e14789.
15. Fidan Ç. **Effects of Ankaferd Blood Stopper on the proliferation of breast cancer cells in vitro.** Master's Thesis. Trakya University Institute of Health Sciences; 2018.
16. Nenni M, Öncül S, Ercan A, Çelebier M, Süslü İ, Haznedaroğlu İC. **Exposure of hepatocellular carcinoma cells to Ankaferd Blood Stopper® alters cell death signaling networks confirmed by oncoproteomic and genomic profiling studies.** *Current Traditional Medicine.* 2021;7:246–258.
17. Buyuktiryaki M, Tayman C, Koyuncu I, Cakir U, Taskin Turkmenoglu T, Cakir E, et al. **Therapeutic and preventative effects of Ankaferd Blood Stopper in an experimental necrotizing enterocolitis model.** *Biomedicine & Pharmacotherapy.* 2019;110:105–110.
18. Koluman A, Akar N, Malkan UY, Haznedaroğlu İC. **Qualitative/Chemical analyses of Ankaferd hemostat and its antioxidant content in synthetic gastric fluids.** *BioMed Research International.* 2016;2016:8957820.
19. Dabak J, Gazuwa S, Ubom G. **Hepatoprotective potential of calcium and magnesium against cadmium and lead-induced hepatotoxicity in Wistar rats.** *Asian Journal of Biotechnology.* 2009;1:12–19.
20. Uboh FE, Ebong PE, Umoh IB. **Comparative hepatoprotective effect of vitamins A and E against gasoline vapor toxicity in male and female rats.** *Gastroenterology Research.* 2009;2:295–302.
21. El Talees AA, Hussien NI, Allam MM, Mohammed DA. **The potential effect of vitamin D on rats with fatty liver induced by a choline-deficient diet.** *Benha Medical Journal.* 2018;35:67–73.
22. Sinbad OO, Folorunsho AA, Olabisi OL, Ayoola OA, Temitope EJ. **Vitamins as antioxidants.** *Journal of Food Science and Nutrition Research.* 2019;2:214–235.
23. Lencioni R, de Baere T, Soulen MC, Rilling WS, Geschwind JF. **Lipiodol transarterial chemoembolization for hepatocellular carcinoma: A systematic review of efficacy and safety data.** *Hepatology.* 2016;64:106–116.
24. Tu J, Jia Z, Ying X, Zhang D, Li S, Tian F, et al. **The incidence and outcome of major complications following conventional TAE/TACE for hepatocellular carcinoma.** *Medicine (Baltimore).* 2016;95:e5606.
25. Muzykantov VR, Brenner JS. **Vascular immunotargeting: Take the highway to the first exit.** *Hepatology.* 2018;68:1672–1674.
26. Avcu F, Guner M, Misirci M, Elci P, Safali M, Goker H, et al. **Evaluation of anti-neoplastic effects of a new hemostatic agent Ankaferd Blood Stopper on myeloma cell line and plasmacytoma development in BALB/c mice.** *Blood.* 2014;124:5728.
27. Ozbek O, Acar K, Koc O, Saritas K, Toy H, Solak Y, et al. **Short-term effects of Ankaferd hemostat for renal artery embolization: An experimental study.** *Cardiovascular and Interventional Radiology.* 2013;36:498–504.
28. Koc O, Acar K, Ozbek O, Guler I, Saritas K, Erdem TB, et al. **Splenic artery embolization with Ankaferd Blood Stopper in a sheep model.** *Diagnostic and Interventional Radiology.* 2016;22:354–357.
29. Tas A, Koklu S, Beyazit Y, Karaca G, Astarci HM, Akbal E, et al. **Percutaneous Ankaferd injection into in vivo liver tissue compared with ethanol in an experimental rat model.** *Clinical Research in Hepatology and Gastroenterology.* 2011;35:549–553.
30. Patıroğlu T, Şahin NE, Ünal E, Kendirci M, Karakükcü M, Özdemir MA. **Effectiveness of Ankaferd Blood Stopper in prophylaxis and treatment of oral mucositis in childhood cancers evaluated with plasma citrulline levels.** *Turkish Journal of Hematology.* 2018;35:85–86.
31. Atay MH, Arslan NA, Aktimur S, Buyukkaya P, Kelkitli E, Turgut M, et al. **Safety and efficacy of Ankaferd Hemostat (ABS) in chemotherapy-induced oral mucositis.** *International Journal of Hematology and Oncology.* 2015;25:166–171.
32. Ozle M, Uğar Cankal DA, Ilhan M, Keleş H, Kupeli Akkol E. **Evaluation of the chemopreventive effects of Ankaferd Blood Stopper in 7,12-dimethylbenz[a]anthracene-induced oral epithelial dysplasia.** *Clinical Oral Investigations.* 2018;22:3091–3096.
33. Metin B, Menevşe E, Sivrikaya A, Altınok T, Arıkoğlu H. **The effects of Ankaferd Blood Stopper on DNA damage and antioxidant enzyme levels in rabbits with pulmonary parenchymal injury.** *Medical Science.* 2017;6:5–10.
34. Uğur A, Saraç N, Cankal DA, Özle M. **The antioxidant and antimutagenic activities of Ankaferd Blood Stopper, a natural hemostatic agent used in dentistry.** *Turkish Journal of Medical Sciences.* 2016;46:657–663.
35. Seyhan Turk, Umit Yavuz Malkan, Mehdi Ghasemi, Helin Hocaoğlu, Duygu Mutlu, Gursel Gunes, Salih Aksu, Ibrahim Celalettin Haznedaroğlu. **Growth inhibitory activity of Ankaferd Hemostat on primary melanoma cells and cell lines.** *SAGE Open Medicine.* 2017;5:1–7.
36. Koçak E, Çelebier M, Haznedaroğlu IC, Altınöz S. **Analysis of the antiproliferative effect of Ankaferd Hemostat on Caco-2 colon cancer cells via LC/MS shotgun proteomics approach.** *BioMed Research International.* 2019;2019:5268031.

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