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ANTI-ANGIOGENIC ACTIVITY ANALYSIS OF THERMAL PROCESSED TURMERIC EXTRACT *IN VIVO* IN DEVELOPING ZEBRA FISH EMBRYOS (DANIO RERIO)

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Abstract: **Turmeric is herbs used as an food adjunct in India, reported to prevent oxidation of fats and oils but the cooking at high temperature is a common practice and the thermal stability of turmeric and its retention of the potential activity are not yet clear. It is recommended in anticancer treatment that they are antiangiogenic through multiple interdependent processes. Angiogenesis plays an important role in the development of cancer and excessive angiogenesis is reported to be related to many diseases. Anti-angiogenic therapy is promising agent for cancer therapy. The scientific validation of anti-angiogenic effects of heat treatment given Standard Turmeric Extract (STE) was done in zebrafish *in-vivo* angiogenesis model. It was observed that the STE molecules inhibited the growth of intersegmental vessel (ISV) of zebrafish embryos in a dose dependent manner, as observed by red blood cells (RBC) staining assay. This study reveals the scientific validation of the potential anti-angiogenic effect of STE supports its use in food preparations as a herbal adjunct for prophylaxis of diseases occur through angiogenesis.**

Keywords: **Thermal Process - Standard Turmeric Extract (STE)- antiangiogenesis- Zebra fish.**

I. INTRODUCTION

Turmeric has been used as a dietary supplement as well as a therapeutic agent in India and in many Asian countries. The herbs wide range of pharmaceutical properties are related to various human metabolic and infectious diseases, diabetes, psoriasis, rheumatoid arthritis, atherosclerosis, Parkinson's and Alzheimer's diseases and several cancers[8,9]. Turmeric has reported to have anti carcinogenic effects on leukemia, skin, cervix, lung, prostate, breast, ovarian, bladder, liver, gastrointestinal tract, pancreatic and colorectal epithelial cancers, lymphomas, multiple myeloma, brain cancer and melanoma(1). The anticarcinogenic effects induced by curcumin in cancer cells are mediated via the modulation of multiple oncogenic signaling transduction elements. Several reports states that the expressions and activities of various oncogenic proteins, inflammatory cytokines and enzymes, transcription factors, and gene-products linked with cell survivals and proliferation, can be modified by curcumin, a the most potent active component of turmeric through participating in the regulation of oncogene protein (2). Curcumin treatment inhibited angiogenesis in a subcutaneous Matrigel plug model in mice and caused the preformed tubes to break down [1]. Turmeric is recommended in anticancer treatment that they are antiangiogenic through multiple interdependent processes (including effects on gene expression, signal processing, and enzyme activities). Although angiogenesis is essential for normal physiological processes, it plays an important role in the development of cancer and excessive angiogenesis is reported to be related to many diseases. Anti-angiogenic therapy is promising agent for cancer therapy. The scientific validation of anti-angiogenic effects of thermal processed Standard Turmeric Extract (STE) was done through evaluation using *in-vivo* zebrafish angiogenesis model. Zebrafish embryo is an excellent model for studying angiogenesis. During early Zebrafish embryogenesis, the

pattern of angiogenesis is simple, primarily occurring in the head and between somite in the trunk. Zebrafish embryo has a similar blood vessels system to mammalian system and can survive several days without blood circulation. Assessment of angiogenesis, apoptosis and toxicity response can be done effectively [8].

2. MATERIALS AND METHODS

Rhizomes of turmeric were obtained from Erode and certified at Tamil Nadu Agricultural University, Coimbatore and powdered All other chemicals were of laboratory grade. Embryos were generated by natural pair wise mating, as described by (12) and staged according to (11). STE was prepared from powder (4) as follows. Turmeric powder (100 g) was mechanically stirred with n-hexane (350 ml) at 25 °C for 24 h. The mixture was filtered and washed thoroughly with fresh n-hexane and dried under the hood overnight. Dried powder was extracted with methanol (350 ml) by stirring mechanically at 25 °C for 24 h, filtered, and washed with methanol. The solvent from the combined filtrate and washings was stripped off and the residue was left under vacuum. The residue was re-dissolved in methanol and the solvent was stripped off. The resulting residue, after leaving under vacuum overnight, was powdered in a mortar and left in vacuum for a minimum of 48 h. The yield of turmeric fraction was 3.61g (3.6%). This Standard Turmeric Extract (STE) dissolved in DMSO was added directly to embryo water. This was subjected for a thermal treatment (pasteurization at 82 ± 2°C for 20 minutes using a hot plate). Twenty four hour post- fertilization zebra fish embryos were incubated with varying concentrations of thermally processed STE continuously for 48 h at 28°C. The final concentration of DMSO was 0.1%. Zebrafish embryos treated with 0.1% DMSO were used as drug carrier control. o-dianisidine staining was used to study the expression of globin (3). On 72 hpf (hours post fertilization) of STE incorporation, embryos were fixed in 4% paraformaldehyde. Dechorionated embryos were stained for 30 min in the dark in o-dianisidine.

3. RESULTS AND DISCUSSION

Turmeric is a naturally herbal supplement in Indian diet possesses anti-oxidative, anti-proliferative, and anti-inflammatory effects (5). Zebrafish has high genetic homology with humans over 85% as well as important parallels in organogenesis and functional mechanisms (4,8) serve as an excellent model organism for studying angiogenesis. Here, Anti-angiogenesis activity of STE was studied in Zebrafish embryos.

Currently, India is the major producer of turmeric, and it is also the major user of its own production. Erode, in Tamil Nadu is one of the largest producer of turmeric centre in the world and the turmeric from this area is reported to be the best quality turmeric. Turmeric biological activities make it a good candidate for development of pharmaceuticals, nutraceuticals, or food ingredients with functional properties. But the quality parameters of the turmeric used in food forms the major criteria for elucidating the effects of the functional properties of various preparations. The quality testing of turmeric with respect to its molecular interactions on host biological system would throw light on its activities like cellular protective, anti carcinogenic, antioxidant and so on. The antiangiogenesis analysis of standard turmeric molecule in the Zebra fish model suggests its therapeutic use in food and feed.

The protese (1mg/ml) treatment resulted in dechorionation of the Zebra fish embryos (Fig1) and these developing embryos were tested for blood vessel formation using RBC staining. Inter segmental vessel formation in developing embryos in various drug concentrations (ranges from 0 to 30 µM) was analyzed. The highest concentration of the STE (30µM) showed edema formation. Hence the lower concentration of 20 µM was taken as optimum. The visibility of blood vessel formation was reported to be clear in 0.1% DMSO treated embryos compared to drug treatment (Fig 2) in staining. The variation in the vessel formation as an identification of anti-angiogenesis property was appreciated in dorsal longitudinal anastomotic vessel, dorsal aorta, posterior cardinal vein and intersegmental vessels.

The present observation supports the concept that turmeric has antitumor activity and it has been found that curcumin suppressed breast tumor angiogenesis by abrogating osteopontin or medroxyprogesterone acetate induced VEGF expression (1,2). The present study supported the report (10) that the antiangiogenic effect of curcumin on endothelial cell migration shown to be the cause for a dose-dependent inhibition of tube formation when the cells were treated before plating or at the time of plating on Matrigel. Curcumin treatment inhibited angiogenesis in a subcutaneous Matrigel plug model in mice and caused the preformed tubes to break down (11,12).

Curcumin inhibited α6β4 signaling and functions by altering intracellular localization of α6β4, and prevented its association with signaling receptors such as the epidermal growth factor receptor (EGFR) and Akt (7). In addition, the

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combination of epigallocatechin gallate (EGCG) and curcumin is efficacious in both *in vitro* and *in vivo* models of ERα-breast cancer. In this processes, the regulation of VEGFR-1 may play a key role in the antitumor activities (6).

A. Figures, Graphs and Tables

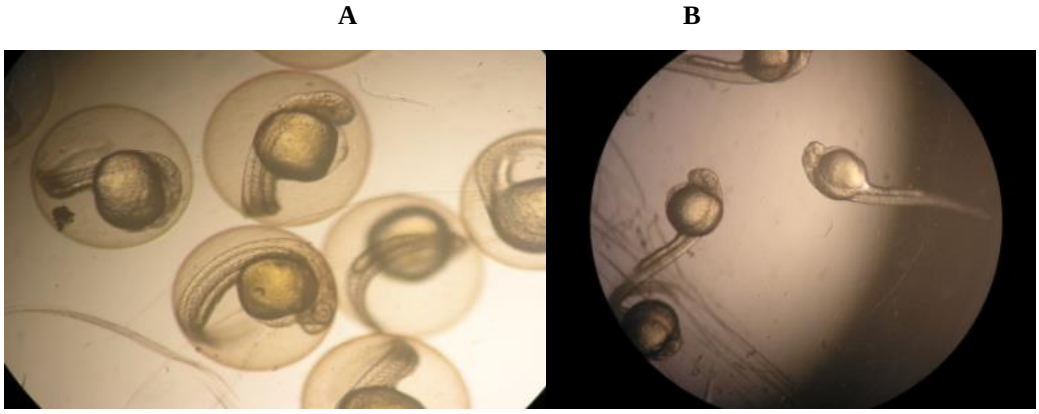

Fig 1 showed the effect of protese ((1mg/ml) treatment in Zebra fish embryos for Dechorionation

A. Before protease treatment

B. After protease treatment

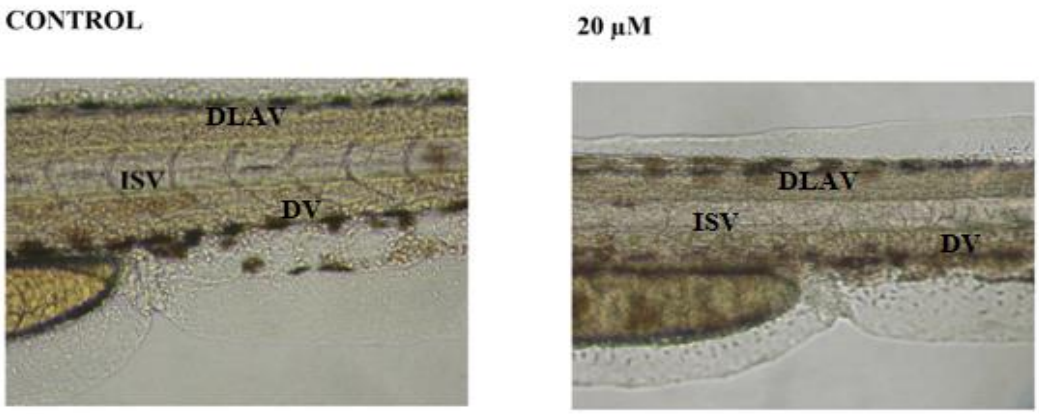

Fig 2: RBC stained zebrafish embryos at 72 hours post fertilization showing vessel formation after drug treatment.

DLAV - dorsal longitudinal anastomotic vessel

DA - dorsal aorta

ISV - intersegmental vessel

4. CONCLUSION

Tamil Nadu is one of the largest producers of turmeric centre in the world and the variet turmeric from this area is reported to be the best quality turmeric. Turmeric biological activities make it a good candidate for development of pharmaceuticals, nutraceuticals, or food ingredients with functional properties. In this study, the potential of thermally processed STE to inhibit the intersegmental vessel formation in zebrafish embryo has been evaluated. This study confirmed the inhibitory effect of STE on blood vessel formation and it was demonstrated that STE have potent anti-angiogenic activity and it was revealed that the potential anti-angiogenic effect of thermally processed STE molecules forms validity for Indian cooking of turmeric and supports the fact that it can be recommended as a food adjunct for therapeutic effect against disease like cancer.

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REFERENCES

[1] Carroll CE, Ellersieck MR, Hyder SM (2008) Curcumin inhibits MPA-in¬duced secretion of VEGF from T47-D human breast cancer cells. Menopause 15:570-4.

[2] Chakraborty G, Jain S, Kale S, Raja R, Kumar S, Mishra R, et al.(2008) Cur¬cumin suppresses breast tumor angiogenesis by abrogating osteopon¬tin-induced VEGF expression. Mol Med Rep 1:641-6.

[3] Iuchi I and Yamamoto M, Erythropoiesis in the developing rainbow trout, Salmo gairdneri irideus: histochemical and immunochemical detection of erythropoietic organs. J Exp Zool. 226: 409

[4] Janet L. F, Janice N.O, Jennifer B.F, Guanjie C, R. Clark et al., (2006) Turmeric Extracts Containing Curcuminoid s Prevent Experimental Rheumatoid Arthritis J Nat Prod. 69: 351–355

[5] Kimmel C B, Ballard W W, Kimmel S R, Ullmann B and Schilling T F (2000) Stages of embryonic development of the zebrafish. Dev Dyn. 203: 253-310.

[6] Lee KW, Kang NJ, Rogozin EA, Kim HG, Cho YY, Bode AM, Lee HJ, Surh YJ, Bowden GT, Dong Z.(2007) Myricetin is a novel natural inhibitor of neoplastic cell transformation and MEK1. Carcinogenesis. 28:1918-27.

[7] Murielle Mand Surinder K(2011). Batra Potential applications of curcumin and its novel synthetic analogs and nanotechnology-based formulations in cancer prevention and therapy Chinese Medicine. 6:31

[8] Somers-Edgar TJ, Scandlyn MJ, Stuart EC, Le Nedelec MJ, Valentine SP, Rosengren RJ(2008).The combination of epigallocatechin gallate and cur¬cumin suppresses ER alpha-breast cancer cell growth in vitro and in vivo. Int J Cancer 122:1966-71.

[9] Soung YH, Chung J (2011). Curcumin inhibition of the functional interaction between integrin α6β4 and the epidermal growth factor receptor. Mol Cancer Ther. 10:883-91.

[10] Thaloor D, Singh AK, Sidhu GS, Prasad PV, Kleinman HK, Maheshwari RK(1998). Inhibition of angiogenic differentiation of human umbilical vein endothelial cells by curcumin. Cell Growth Differ. 9(4):305-12.)

[11] Vogel, A and Weinstein B (2000) Studying vascular development in the zebrafish.TCM 10: 847.

[12] Westerfield, M.(2000). The Zebra fish Book. Guide for the Laboratory Use of Zebrafish (Danio rerio) (University of Oregon Press, Eugene), 4th Edition.