EFFECT OF SODIUM GLUTAMATE MONOHYDRATE ON ANGIOGENESIS - ZEBRAFISH (DANIO RERIO) AS A MODEL ON EARLY DEVELOPMENTAL STAGE

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Abstract- Glutamate, an important amino acidpresent in variousdiets and normally present in the form of Sodium glutamate monohydrate (SGM) salt and commonly known as Monosodium glutamate, a sodium salt of L-glutamic acid. It is naturally occurring and non-essential salt and also used as the main flavour enhancers in various food products and they are associated with Chinese restaurant syndrome. Several approaches have been taken to investigate putative receptors for SGM including taste buds, animal behavioural studies, electrophysiological recordings, Ca2+-imaging and molecular biological experiments using several animal models.Zebrafish, Danio rerio, provides an excellent system to study developmental biology in investigating a wide range of compounds including food additives, flavour enhancer, neurotoxicity of drugs, environmental chemicals. In this present study, we have used zebrafish embryos to investigate angiogenic modulating role of SGM during development. Zebrafish embryos at 24hours post fertilization (hpf) were exposed to different concentrations of SGM 50µM and 100µM in Hank's embryo medium maintained at 28 ± 2°C. Morphometric changes, hatching rate, survival rate and heartbeat was monitored and document using light microscope, at 48hpf and 72hpf. Embryo growth i.e., body length was measured using captured image with Image J software. Real-time PCR was performed for gene expression of key players in angiogenesis: VEGF, VEGFR2, NRP1, HIF1a, Adenosine receptors (A1, A2a.1, A2a.2 and A2b), angiopoietin, Ephb4. Data were expressed as mean ± SEM. Statistical analyses were performed using one-way ANOVA followed by Tukey's Multiple Comparison tests using Graph Pad Prism version-6 software.SGM treated embryo showed mild to severe pericardial edema with increased heartbeat, significant increase in hatching rate respective to 48hpf and 72hpf, compared to control. While survival decreased significantly with time and with higher dose of 100µM at 72hpf. Increased growth of embryos were observed with 50µMand 100µM concentration. VEGF-A, VEGFR2, NRP1, HIF1a, Adenosine receptors (A1, A2a.1, A2a.2 and A2b), angiopoietin, Ephb4 showed significantincreased gene expression. VEGF-A, VEGF R2, Adenosine receptor showed 1.25 to 1.65-fold increase in SGM treated embryos at 72hpf with increased mortality.Result obtained commends the involvement of SGM in modulating angiogenesis and growth during the early developmental stage,suggesting that high dose or long term exposure in food might be toxic to offspring and disrupt endocrine function. Further experiments are needed to test if SGM contributes in some manner to glutamate receptor signalling in angiogenesis. Dosage and time of exposure to SGM has to be more concerned to avoid multiple disorders during human lives.

Keywords- Sodium glutamate monohydrate, zebrafish, embryo, development, angiogenesis.