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The Effect of Administration of Nano-Curcumin on the Level of Vascular Endothelial Growth Factor (VEGF) Expression and Fetus Length in Pregnancy of Mice under Stress Conditions

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ABSTRACT

Environmental noise is an important factor that contributes to maternal stress during pregnancy. Vascular Endothelial Growth Factor (VEGF) is an important angiogenesis factor that plays a major role in maintaining homeostasis and fetal development. In the medical field, nano-curcumin is often used for its antioxidant and anti-inflammatory properties. This study used 25 mice that were allotted 5 treatment groups: K(-) with no treatment, K(+) with an induced disturbance of 135dB for 40min with a continuous pattern, P1, P2, and P3 were given a disturbance of 135dB for 40min with a continuous pattern and nano-curcumin doses of 14, 21, and 24.5mg/kg BW, respectively. The Post Hoc Bonferroni Test was applied to determine real differences between each treatment and analysis of fetal length data using Pairwise Comparison tests. The result analysis revealed that treatment 3 (135dB noise for 40 minutes continuously @ concentration of 24.5 nano-curcumin) showed a decrease in VEGF. It was concluded that noise treatment of 135dB for 40 minutes in a continuous pattern with a dose of 24.5mg/kg BW leads to a decrease in expression of VEGF as compared to the negative control. Treatment with 135dB noise for 40min in a continuous pattern with doses of 21 and 24.5mg/kg BW showed an increase in fetal length compared to the positive control.

Key words: VEGF, Nano-curcumin , Noise, Fetus length

INTRODUCTION

Fetal growth and development are greatly influenced by the nutritional intake received by the fetus. Nutrients are distributed to the fetus through the mother's placenta (Suciyati et al. 2020). Stress generated by disorders can cause the release of stress hormones such as catecholamines and glucocorticoids. Stress that occurs during pregnancy can have a negative impact on fetal development, this could be due to the narrowing of the placental arteries and the obstruction of the flow of nutrients and oxygen to the fetus (Jafari et al. 2017).

Vascular Endothelial Growth Factor (VEGF) is an important mediator or regulator in the process of forming new blood vessels (angiogenesis) which is produced in the placenta to ensure oxygen transport via blood smoothly (Gondo and Haryanti 2020). The effect of VEGF is usually to increase vascularization and cell survival (Scheiner et al. 2019).

Curcumin is a polyphenolic compound extracted from *Curcuma longa* L. (Dutta et al. 2021; Dahab et al. 2023). This medicinal plant is known for its contents such as antioxidants, anti-inflammatory, antifungal, and neuroprotective. Based on pharmacokinetic properties, the efficacy of curcumin shows quite low bioavailability. Therefore, an approach was taken to increase the bioavailability of curcumin by formulating nanoparticles (Dutta et al. 2021). Formulating curcumin into nanoparticles can increase bioavailability and solubility in tissues, thereby providing fast and optimal therapeutic effects (Sandhiutami et al. 2022). Trials in humans and mice have shown that oral consumption of curcumin shows lower levels of bioavailability and accumulates in the intestine. This curcumin barrier can be removed by the synthesis of curcumin nanoparticles (nano-curcumin), which can be used to prolong circulation, improve permeability and increase resistance to metabolic processes

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(Pandit et al. 2015). According to Tuong et al. (2023), nano-curcumin @ 10mg/kg BW could have a slight effect on animals. Therefore, in this research, we tried to increase the dose and see whether it could still have a good effect.

MATERIALS AND METHODS

Ethical approval

This research approval was obtained from the Research Ethics Commission of Brawijaya University (NO: 028-KEP-UB-2023).

Experimental design

The research was conducted at the Airlangga University Laboratory and the Healthy Animal Laboratory. A completely randomized block design was carried out for 5 treatment repetitions. The treatment given consisted of 5 groups, namely K- in the form of a group of pregnant mice without any treatment; K+ was a group of pregnant mice that were given noise of 135dB for 40min with a continuous pattern; treatment 1 (P1) consisted of a group of pregnant mice that were given 135dB noise for 40min with a continuous pattern and given a dose of 14mg/kg BW of nano-curcumin; treatment 2 (P2) consisted of a group of pregnant mice given 135dB noise for 40min with a continuous pattern and given a dose of 21mg/kg BW of nano-curcumin; treatment 3 (P3) consisted of a group of pregnant mice that were given 135dB noise for 40min in a continuous pattern and given a dose of 24.5mg/kg BW of nano-curcumin. Treatment was carried out from days 6 to 18 of pregnancy.

Experimental mice

The procedure began by inducing female mice using the Pregnant Mare Serum Gonadotropin hormone (PMSG or Foligon) (Product code 20000029, manufacturer: MSD Saúde Animal, Boxemer, Belanda) @ 5IU/0; 1mL/animal. After 48h, the hormone human Chorionic Gonadotropin (HCG or Chorulon) (Product code: 11466175, manufacturer: MSD Saúde Animal, Germany) at a dose of 5IU/0, 1mL/animal was injected and immediately mated with male mice by polymatting. After 17h of mating, the female mice were examined for vaginal plugs. The appearance of the vaginal plug was used as a benchmark for pregnancy at day zero.

Immunohistochemical staining of the placenta

Endothelial VEGF expression was observed microscopically at 400x in 10 fields. VEGF staining was carried out using immunohistochemical techniques with specific monoclonal antibodies. Calculation of VEGF expression in immunohistochemical staining was carried out using the Allred Scoring System (Table 1), namely the sum of the "proportion score" and "intensity score" of cells that transmit VEGF (Bintari dan Yuliani 2020). In the staining process, deparaffinization was carried out by heating at 60°C for 60min. The preparations were processed in xylol, ethanol 90, 80, 70%, and absolute ethanol for 5min each. Then, the preparation was washed with sterile distilled water 3 times in 5min. Antigen retrieval with citrate buffer and IHK staining with anti-VEGF primary antibody and incubation for 18h. secondary antibody incubation for 30min, SA-HRP incubation for 30min, application of DAB chromogen, counterstain with Mayer hematoxylin, and mounting with entellan (Subiantoro et al. 2020).

The length of the fetus

Fetal measurements were carried out after the expulsion of the fetus at 18 days of gestation and soaked with 96% alcohol. Fetal length was carried out by direct measurement using a plastic ruler from the tip of the nose to the distal end of the tailbone (Stephens et al. 2015). Observed fetal morphology, including the spine, ribs, stenebrate, and metacarpus (Prasetyawati et al. 2017).

Statistical analysis

This study's parameters were VEGF expression and fetal length development. Data were analyzed quantitatively using the SPSS software expression using the parametric ANOVA test, followed by further testing in the form of a post-application with a confidence level of 99%. VEGF Hoc Test using the Bonferroni Test. Meanwhile, the development of fetal length uses the non-parametric Kruskal-Wallis test. The results obtained about fetal length were analyzed using SPSS software with a normality test ($P<0.01$). Kruskal-Wallis test (nonparametric test) was used to show normality results ($P<0.01$) so the data was not normal.

RESULTS

Effect of nano-curcumin administration on VEGF expression in the placenta of pregnant mice under stress conditions

The negative control group had the lowest mean value of VEGF expression (Table 2), namely (18.44%) and had a significant difference to the positive control group without treatment (31.85%) and P1 with 135dB noise for 40min with a continuous pattern and a concentration of 14 nano-curcumin (30.96%) and had no significant difference to the P3 group with 135dB noise for 40min pattern and a concentration of 24.5mg/kg BW nano-curcumin (19.63%). In the nano-curcumin administration group (Table 2), the lowest mean percentage of VEGF expression was in the P3 group with 135dB noise for 40min continuous pattern and a concentration of 24.5mg/kg BW nano-curcumin (19.63%).

The image below (Fig. 1) shows the expression of VEGF using immunohistochemical staining techniques. VEGF expression is visualized with brown areas. This brown color is the expression of cytotrophoblast cells and endothelial cells which are parameters for calculating VEGF expression (Hendrawan et al. 2017). The results of VEGF expression in mouse placental cells can be seen in Fig. 1.

Effect of nano-curcumin administration on fetal length in pregnant mice under stress conditions

The results of the Pairwise Comparison test for fetal length (Table 3) parameters showed ($P<0.01$). The results of the Kruskal-Wallis test showed that group P1, with 135dB noise for 40min in a continuous pattern and a concentration of 14 nano-curcumin, had the lowest average fetal length, 1.70±0.32. The P3 group with 135dB noise for 40min continuous pattern and a concentration of

Table 1: Scoring system by Remmele and Stegner (IRS, Immunoreactive Score)

Score	Percentage of Positively Stained Cells (PP)	Intensity of Staining (SI)	IRS Points (PP x SI)	IRS Classification
0	no staining	no color reaction	0-1	Negative
1	<10	weak reaction	2-3	Positive, weak expression
2	10-50	moderate reaction	4-8	Positive, moderate expression
3	51-80	strong reaction	9-12	Positive, strong expression
4	>80			

Table 2: Results of examination of VEGF expression in the placenta

Sample	Comparison	Mean VEGF (%)	Mean±SD
K(-)	K(+)	18.44	25.15±7.83
	P1		24.70±7.55
	P2		22.65±5.82
	P3*		19.04±3.50*
K(+)	P1	31.85	31.41±2.85
	P2		29.36±3.95
	P3		25.74±6.91
P1	P2	30.96	28.91±3.93
	P3		25.30±6.63
P2	P3	19.63	23.25±4.86

Asterisk indicates non-significant difference compared to the negative control with a significance value of 1%. K- received no treatment, K+ with noise induction 135dB for 40min continuous pattern, P1 noise induction 135dB for 40min continuous pattern and nano-curcumin 14mg/kg BW, P2 noise induction 135dB for 40min continuous pattern and nano-curcumin 21mg/kg BW, and P3 noise induction 135dB for 40min continuous pattern and nano-curcumin 24.5mg/kg BW. Measurements using immunohistochemistry by looking at the intensity of the brown color on the preparation.

Table 3: Results of the pairwise comparison test for fetal length (mm)

Sample	Comparison	Mean±SD
K(-)	K(+)	2.03±0.26
	P1	1.89±0.35
	P2*	2.15±0.22*
	P3*	2.01±0.28*
K(+)	P1	1.82±0.29
	P2*	2.07±0.23*
	P3*	1.93±0.24*
P1	P2	1.94±0.34
	P3	1.80±0.29
P2	P3*	2.05±0.25*

Asterisk shows no significant difference compared to the negative and positive control groups with the Pairwise Comparison level having a value of 1%. K- received no treatment, K+ with noise induction 135dB for 40min continuous pattern, P1 noise induction 135dB for 40min continuous pattern and nano-curcumin 14mg/kg BW, P2 noise induction 135dB for 40min continuous pattern and nano-curcumin 21mg/kg BW, and P3 noise induction 135dB for 40 min continuous pattern and nano-curcumin 24.5mg/kg BW. Measurements using a plastic ruler from the nose to the tail of the fetus

24.5mg/kg BW nano-curcumin had a value of 1.96±0.32 which had no significant difference to the negative control group (2.03%). In the group that gave nano-curcumin, the average percentage of fetal length in the P3 group with 135dB noise for 40min with a continuous pattern and a concentration of 24.5mg/kg BW nano-curcumin (1.96%) was less than significantly different (P<0.01). treatment group P1 with 135dB noise for 40min continuous pattern and a concentration of 14mg/kg BW nano-curcumin and P2 with 135dB noise for 40min continuous pattern and a concentration of 21mg/kg BW nano-curcumin.

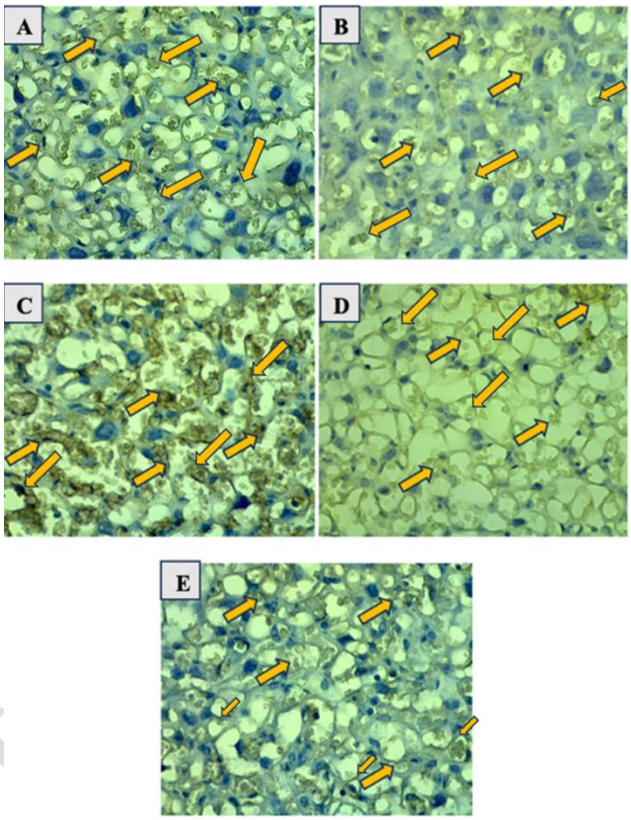

Fig. 1: Results of immunohistochemical preparations for VEGF expression (orange arrow) in the placental organ preparations of pregnant mice (400x); A) K- group without treatment; B) K+ group with noise 135dB for 40min continuous pattern; C) P1 with noise of 135dB for 40min continuous pattern and nano-curcumin dose of 14mg/kg BW; D). group P2 with noise 135dB for 40min continuous pattern and nano-curcumin dose of 21mg/kg BW; and E) P3 with noise of 135dB for 40min continuous pattern and nano-curcumin dose of 24.5mg/kg BW.

DISCUSSION

The placenta is an important tissue that has a relationship between hypoxia (low O_2 pressure), organogenesis (organ development), and angiogenesis (blood vessel development). Noise in pregnant mice causes significant side effects such as organ dysfunction, uteroplacental insufficiency, and fetal growth retardation (decreased fetal length). Changes in the disturbed spiral arteries cause placental hypoxia and reduce fetal blood circulation. Abnormal development of placental villi decreased the proliferation of villous and extra villous cytotrophoblasts, and insufficient placental implantation led to trophoblast invasion. Trophoblasts produce VEGF and other angiogenic factors that support placental vasculature by paracrine and autocrine actions. Decreased VEGF and increased production of FMS-like tyrosine-containing kinase-1 (sFLT-1) negatively affect placental vascular development (Özgökçe et al. 2023).

Normal noise exposure is 85dB, while exposure to noise intensity of more than 115dB can cause direct mechanical trauma to ear cells and tissues (Jongkamonwiwat et al. 2020). When inflammation of the organ of Corti occurs, the body responds by releasing inflammatory cytokines or chemokines (Fujioka et al. 2014). Apart from cytokines secreted during inflammation in the organ of Corti, there are also hydroxyl radicals carried by the blood to the placenta, thereby affecting the permeability of cell membranes. An imbalance in cell membrane permeability can cause an increase in blood viscosity resulting in a decrease in oxygen supply to the fetus (Hendrawan et al. 2017). The accumulation of macrophages in the circulation triggers the release of VEGF, which is an important regulator in the process of forming new blood vessels (angiogenesis) (Gondo and Haryanti 2020). To maintain balance and repair blood vessels due to inflammation, supplementation therapy is needed. Nano-curcumin which comes from turmeric rhizomes has several chemical contents, including tannins and flavonoids which can act as antioxidants can prevent cell damage caused by free radicals (Prabowo et al. 2019). In addition, vitamin C or ascorbic acid contained in nano-curcumin is a strong antioxidant compound that not only interacts directly with ROS but also contributes to the regeneration of other antioxidants.(Permatananda et al. 2020).

Conclusion

Based on the results, it shows that treatment 3, which was given 135dB noise for 40min continuously at a concentration of 24.5mg/kg BW nano-curcumin, showed a decrease in VEGF. The results of treatment 2 were given 135dB noise for 40min with a continuous pattern at a concentration of 21mg/kg BW and treatment 3 was given 135dB noise for 40min with a continuous pattern at a concentration of 24.5mg/kg BW nano-curcumin, showing an increase in fetal length compared to the group positive control.

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Authors' contribution

Viski Fitri Hendrawan: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Writing Original Draft, Visualization, Supervision, Project Administration, Funding Acquisition. Galuh Chandra Agustina: Methodology, Validation, Investigation, Resources. Gretania Residiwati: Methodology, Validation, Investigation, Resources. Marwah Azrianti Maulidia Amir: Writing Original Draft, Visualization, Writing, Review and Editing.

Conflict of interests

The authors declare no conflict of interest.

REFERENCES

Bintari IG and Yuliani MGA, 2020. Deteksi Aeromonas hydrophila pada Ginjal Mencit *Mus musculus* dengan Teknik Imunohistokimia. Jurnal Agriektensia 19(2): 114-121.

Dahab MAE, Sayed A and Mahana N, 2022. Curcumin impact on ex vivo *Toxocara vitulorum* adult worms and eggs. International Journal of Veterinary Science 11(3): 280-288. https://doi.org/10.47278/journal.ijvs/2021.122

Dutta A, Patil RK and Pati HCI, 2021. Curcumin: Its bioavailability and nanoparticle formulation: A review. International Journal of Health Sciences and Research 11(10): 228–238. https://doi.org/10.52403/ijhsr.20211030.

Fujioka M, Okano H and Ogawa K, 2014. Inflammatory and immune responses in the cochlea: Potential therapeutic targets for sensorineural hearing loss. Frontiers in Pharmacology 5: 287. https://doi.org/10.3389/fphar.2014. 00287

Gondo HK and Haryanti E, 2020. Pengaruh Spirulina Terhadap VEGF Dari Trofoblast Tikus Putih Hamil Strain Wistar Yang Diinduksi Interleukin 6. Jurnal Ilmiah Kedokteran Wijaya Kusuma 9(2): 123. https://doi.org/10.30742/jikw.v9i2.750

Hendrawan VF, Widjiati W, Susilowati S and Srianto P, 2017. Peningkatan Ekspresi Vascular Endothel Growth Factor Dan Mitogen Activating Protein Kinase Plasenta Tikus Yang Dipapar Carbon Black. Jurnal Veteriner 18(1): 59–68. https://doi.org/10.19087/jveteriner.2017.18.1.59

Jafari Z, Mehla J, Kolb BE and Mohajerani MH, 2017. Prenatal noise stress impairs HPA axis and cognitive performance in mice. Scientific Reports 7(1): 10560. https://doi.org/10.1038/s41598-017-09799-6

Jongkamonwiwat N, Ramirez MA, Edassery S, Wong ACY, Yu J, Abbott T, Pak K, Ryan AF and Savas JN, 2020. Noise exposures causing hearing loss generate proteotoxic stress and activate the proteostasis network. Cell Reports 33(8): 108431. https://doi.org/10.1016/j.celrep.2020.108431

Özgökçe Ç, Öcal A, Ermiş IS and Deveci E, 2023. Histopathological, ultrastructural, and immunohistochemical examination of changes in the placenta as a result of severe preeclampsia. Acta Cirurgica Brasileira 38: e382023. https://doi.org/10.1590/acb382023

Pandit RS, Gaikwad SC, Agarkar GA, Gade AK and Rai M, 2015. Curcumin nanoparticles : Physico-chemical fabrication and it's in vitro efficacy against human pathogens. Biotech 5: 991-997.

Permatananda PANK, Aryastuti AASA, Cahyawati PN, Udiyani DPC, Wijaya D, Pandit IGS and Wirajaya AANM, 2020. Phytochemical and antioxidant capacity test on turmeric extract (*Curcuma longa*) traditionally processed in Bali. Jurnal Bali Membangun Bali 1(2): 135–142. https://doi.org/10.51172/jbmb.v1i2.115

Prabowo H, Cahya IAPD, Arisanti CIS and Samirana PO, 2019. Standarisasi Spesisifik Dan Non-Spesifik Simplisia Dan Ekstrak Etanol 96% Rimpang Kunyit (Curcuma Domestica Val). Jurnal Farmasi Udayana 8(1): 29–35. https://doi.org/10.24843/JFU.2019.v08.i01.p05

Prasetyawati F, Gofur A and Lestari U, 2017. Skeletal malformation on Balb-C foetal mice *(Mus Musculus)* administered by immature pineapple fruits extract. KnE Life Sciences 3(4): 86. https://doi.org/10.18502/kls.v3i4.691

Sandhiutami NMD, Dewi RS, Khairani S and Widyadari SAM, 2022. Evaluasi Keamanan Dari Pengembangan Formula Nanopartikel Kurkumin Pada Mencit Dan Potensi Antioksidan In-VitroEvaluasi Keamanan Dari Pengembangan Formula Nanopartikel Kurkumin Pada Mencit Dan Potensi Antioksidan In-Vitro. Jurnal Ilmu Kefarmasian Indonesia 20(1): 63. https://doi.org/10.35814/jifi.v20i1.1187

Scheiner KC, Maas-Bakker RF, Nguyen TT, Duarte AM, Hendriks G, Sequeira L, Duffy GP, Steendam R, Hennink WE and Kok RJ, 2019. Sustained release of vascular endothelial growth factor from poly(ε-Caprolactone-PEG-ε-Caprolactone)- *b* -Poly(L-Lactide) multiblock copolymer microspheres. ACS Omega 4(7): 11481–11492. https://doi.org/10.1021/acsomega.9b01272

Stephens RB, Karau KH, Yahnke CJ, Wendt SR and Rowe RJ, 2015. Dead mice can grow – variation of standard external mammal measurements from live and three postmortem body states. Journal of Mammalogy 96(1): 185–193. https://doi.org/10.1093/jmammal/gyu022

Subiantoro A, Utariani A and Susilo I, 2020. Expression of vascular endothelial growth factor (VEGF) and new blood vessels formation on wound incision post ropivacaine administration in animal model. International Journal of Innovative Science and Research Technology 5(7): 829–836. https://doi.org/10.38124/IJISRT20JUL550

Suciyati NA, Nurcahyani N, Sutyarso S and Rosa E, 2020. Normality of the mice's fetal spine during fennel flower extract (*Nigella sativa*) feeding. Jurnal Ilmiah Biologi Eksperimen Dan Keanekaragaman Hayati 7(1): 41–47. https://doi.org/10.23960/jbekh.v7i1.14

Tuong DTC, Moniruzzaman M, Smirnova E, Chin S, Sureshbabu A, Karthikeyan A and Min T, 2023. Curcumin as a potential antioxidant in stress regulation of terrestrial, avian, and aquatic animals : A review. Antioxidants 12: 1700. https://doi.org/10.3390/antiox12091700