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Research Article

Venlafaxine induces Teratogenesis and alters SHH Gene Expression and Protein Biochemistry of Developing *Gallus* sp. Embryos

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Abstract

The use of antidepressant drugs during pregnancy is increasing globally. SNRIs and SSRIs are most widely used for treating panic disorders. Venlafaxine is an antidepressant that inhibits serotonin and norepinephrine reuptake. Given the increasing prevalence of antidepressant use during pregnancy, Venlafaxine exposure during the early stages of development could lead to changes in protein dynamics and disrupt essential gene pathways, raising concerns about the potential teratogenic effects of antidepressant exposure during pregnancy. This study explores the developmental impact of Venlafaxine, a commonly prescribed serotonin-norepinephrine reuptake inhibitor (SNRI), on protein biochemistry and gene expression in the developing *Gallus gallus domesticus* embryos. Significant changes in protein profiles were observed among control and Venlafaxine-treated groups. Sonic Hedgehog (SHH) gene, a key regulator of embryonic patterning and organogenesis, specifically neural tube formation, and limb development, is known for its crucial role in embryonic development. qRT-PCR analysis of Venlafaxine-treated embryos showed changes in SHH gene expression suggesting that Venlafaxine may target SHH gene expression, and potentially alter signalling pathways crucial for normal embryogenesis. However, detailed studies are needed to understand the long-term consequences of prenatal and foetal antidepressant exposure on embryonic growth and development. These changes may lead to abnormal growth patterns or congenital defects, emphasizing the need for caution when prescribing Venlafaxine during pregnancy.

Keywords: Antidepressants, Venlafaxine, SNRI, *Gallus gallus domesticus*, Sonic Hedgehog (SHH), Protein Biochemistry, qRT-PCR

INTRODUCTION

The long-term treatment of acute psychosis, bipolar disorder, and depression primarily involves the use of antipsychotics. They can be categorized based on their ability to cause extrapyramidal symptoms (EPS) as first-generation (typical) and second-generation (atypical) antipsychotics. Second-generation antipsychotics when administered may result in adverse metabolic effects. [1] The mode of action of antidepressants works by increasing norepinephrine and serotonin in the synaptic cleft at both supraspinal and spinal levels by reinforcing descending inhibitory pathways. [2] Depending upon the duration of the treatment, second-generation antidepressants like Duloxetine, Venlafaxine, Fluoxetine, Escitalopram, Sertraline, Carbamazepine, and Imipramine, are known to cause hepatotoxicity by induced liver injury through their adverse reactions in the body. Antidepressant drugs also have some remarkable impact on epidemiological and genetic information. [3] Extensive research has shown that antipsychotics can cross the placenta and cause an effect on the developing foetus. [4] Venlafaxine is a structurally novel phenylethylamine antidepressant belonging to the Serotonin- Norepinephrine Reuptake Inhibitor (SNRI) group, with little or no affinity for muscarinic, histaminergic, or adrenergic receptors. [5] It is a second-generation antidepressant used in treating general anxiety disorder (GAD) and major depression. [6] Venlafaxine has appeared to be a successful post-SSRI era antidepressant which is relatively more efficient in reducing major depression. It can also be used in treatment-resistant depression (TRD), especially when SSRIs fail. Venlafaxine functions to inhibit serotonin and norepinephrine reuptake and can also work as a mild inhibitor of dopamine. [5] Venlafaxine regulates stress response by acting on the immune system. [7] Studies have shown that Venlafaxine limits renovascular hypertension, which leads not only to endothelial dysfunction but also serum oxidative and nitrosative stress, and brain and aortic oxidative stress. Venlafaxine can inhibit superoxide generation and prevent oxidative and nitrosative stress involved in the pathogenesis of depression. [8] The liver extensively metabolizes and

absorbs Venlafaxine by the cytochrome P450 2D6 enzyme system. [9]

The phenomenon that results in malformations or birth defects in a foetus or embryo is called teratogenesis. An external substance known as a teratogen is responsible for causing these deformations. [10] There are 2 types of mechanisms based on congenital malformations: i) interactions of an embryo with environmental factors during gastrulation (radiations, drugs, chemicals) and ii) genetic programming inaccuracies depending on deviations in the genotype. [11] Of all congenital malformations approximately 7% are caused by teratogenic agents, which are also responsible for 4-6% of birth defects. Structural abnormalities like anencephaly, ventricular septal defect (VSD), dysmelia, and cleft palate can be caused due to exposure to teratogens. [4] *Gallus gallus domesticus,* commonly known as domestic chicken is a remarkably advantageous model organism for experimental embryology, teratology, and developmental biology. Since the foetus is less vulnerable to morphologic alterations when the maturation of most of the organs occurs, anomalies connected to exposure to teratogenic agents during the foetal stage results in abnormalities during embryogenesis. Additional effects include prenatal onset growth restriction, infertility, structural defects, functional CNS anomalies, miscarriage, or even foetal death. [12]

The Yolk Sac Membrane (YSM) is a bilaminar omphalopleure consisting of a thin, exterior layer of extraembryonic ectoderm and a layer of large yolk-filled cells. It arises from the Area Opaca of the blastoderm at Hamburger Hamilton (HH) stage 24-25. The yolk sac is majorly involved in early hematopoiesis and blood vessel formation, along with contributing to passive immunity by transferring maternal antibodies (IgY) through the developing egg. [13] It also facilitates nutrition supply and gaseous exchange to the developing embryo, as CAM is not developed by then. [14] Any perturbations in its vasculature therefore severely affect embryogenesis. The absorption of yolk is done by phagocytosis by the extraembryonic endoderm. The yolk sac membrane is neither functionally nor morphologically part of the embryonic gut. [15]

SDS-PAGE is a commonly used technique for the qualitative analysis of proteins. It is based on isotachophoresis which is exhibited by the addition of stacking gel onto resolving gel. Sodium Dodecyl Sulphate (SDS) and a Dithiothreitol (DTT) reagent denatures protein samples by breaking disulfide bonds when samples are heated at 95°C. An equal quantity of SDS binds with polypeptide chains irrespective of the type of amino acids present. Proteins form a complex with SDS, impart a negative charge, and get separated based on their molecular weight. [16]

Sonic Hedgehog (SHH) is a gene that regulates the growth and development of the midbrain, forebrain, neural tube, tube isthmus, wing mesenchyma, retina, limb buds, somites, and ectoderm. [17] SHH signalling is critical in various developmental and repair processes across multiple organ systems. It induces endothelial and connective tissue support cells to release proangiogenic factors such as Ang1, Ang2, and VEGF, thereby influencing vasculogenesis and angiogenesis. In the nervous system, SHH is essential for late neuronal differentiation of the forebrain and orchestrates cerebellar development and maturation, including fissure formation. In the liver, SMO protein in the SHH pathway promotes adult liver repair through epithelial–mesenchymal transition. At late organogenesis, it is crucial for the proper development of smooth muscle in the gallbladder. It is also critical for the proliferation and maturation of the pancreas. SHH plays an important role in regulating the development, differentiation, homeostasis, and neoplastic transformation of epithelia in the gastrointestinal tract. It is necessary for the growth and differentiation of the oesophagus. In addition, SHH plays an important role in the development of the lung, specifically in lung specification, formation of the primary bud, and branching morphogenesis. [18] The GLI transcription factor family comprises GLI1, GLI2, and GLI3; the SHH pathway acts on gene expression through its signalling activity. [19] The SHH pathway is distinctly categorized into canonical and non-canonical pathways. The canonical SHH signal transduction pathway comprises many important components. One is the Patched receptor (PTCH1, PTCH2), a 12-domain transmembrane receptor. The Smoothened receptor (SMO) is another 7-domain transmembrane receptor coupled to the G protein-coupled receptor (GPCR), and negative regulatory protein suppressor of fused homolog (SUFU) and the Glioma-associated oncogene homolog (GLI) family of transcription factors (GLI1, GLI2, and GLI3). SHH ligands promote the expression of SMO by PTCH.

Venlafaxine Extended-Release is effective for treating major depressive disorders and anxiety disorders in double-blind and placebo-controlled clinical trials. [20] Venlafaxine blocks the Norepinephrine (Noradrenaline) transporter in the human brain as seen in patients with MDD. [21] Duloxetine Hydrochloride and Venlafaxine Hydrochloride Extended-Release have been used in the treatment of MDD, with Venlafaxine showing a higher affinity for serotonin than norepinephrine transporters. [22] After the 10-week trial, studies reported that with the same dose, significant differences were noted in total PDSS (Panic Disorder Severity Scale) change ($p \leq 0.05$) beginning at week 4 with significantly more Venlafaxine-XR patients reaching response at week 4 than those in the placebo group. [23] According to an advisory on potential adverse effects of SSRIs and other antidepressants on newborns, reports described symptoms such as breathing difficulties, seizures, muscle rigidity, and constant crying. [24]

Serotonin and norepinephrine are expressed quite early on during embryogenesis, therefore influencing early gastrulation. These neurotransmitters are believed to be acting as morphogens, which are signalling molecules having a dose-dependent function on receptive cells. Various evidences from animal studies indicate serotonin acts as a morphogen in craniofacial and cardiac development. There are evidences of norepinephrine influencing neural crest cell formation and differentiation during embryogenesis. In conclusion, the

effect of Venlafaxine during early pregnancy and its probable interference with embryological signalling pathways suggests that it could affect craniofacial and cardiac development. [25]

MATERIALS AND METHODS

Fertilized and pre-incubated *Gallus gallus domesticus* eggs of 4-5 days or HH stage 24-25 were obtained from Venkateshwara Hatcheries Pvt. Ltd. Pune. Embryos were divided into control and treated groups (n=10, *3 sets). Sterile freshly prepared solution of 5mM and 10mM Venlafaxine (Intas Pharmaceuticals) was administered by *in-ovo* (air sac route) technique into the developing 4-5 day old chick embryos (n=10) and on the YSM vessels of 4–5 day old embryos (n=10). Control and treated embryos were incubated for 24 hours at 37°C and 70-80% relative humidity in a BOD incubator. Control and treated embryos were isolated in 1X sterile chilled Phosphate-Buffered Saline (PBS) of pH 7.4. Drug-induced teratogenesis was observed with a dissecting binocular Stereo Zoom microscope (Magnus MAZ Series)

YSM Assay- To evaluate the effect of Venlafaxine on angiogenesis, the YSM assay was performed on 4–5 day old embryos. The development of the YSM vascular network was visualized on embryonic day 5. YSM analysis was done using Wimasis software. The YSM vessels of the 4-5 day old control and treated (5mM and 10mM Venlafaxine) embryos were observed for morphological changes. [26]

Protein Biochemistry- Control and Venlafaxine-treated embryos were homogenized in 1X Protein Extraction Buffer (PEB) at 4°C. The homogenates were vortexed thoroughly and centrifuged at 2000-3000 RPM for 5 minutes at 4°C. The supernatant was used for protein estimation by using Bradford's Assay. [27] SDS-PAGE was used to qualitatively analyse the proteins isolated from the 4-5 day old control and Venlafaxine-treated embryos. [28]

Molecular Biology and Genomic Studies- RNA isolation from control and Venlafaxine-treated embryos was performed using TRIzol®. Primers were designed for PCR analysis using PrimerQuest [29] and oligoanalyzer. RNA Extraction Kit (HiGenoMB, catalogue number MB602) was used to extract total RNA from control and Venlafaxine-treated embryos. RNA quantification was done spectrophotometrically. The purity of the RNA sample was measured by taking the A260/A280 ratio. After determining RNA purity and estimating RNA integrity number (RIN), mRNA was reverse transcribed into cDNA using cDNA reverse transcription kit (Applied Biosystems, catalogue number 4368814).

Quantitative Real-Time PCR (qRT-PCR)- The target gene (SHH) expression analysis was performed by SYBR green chemistry detection with Quantstudio 5 Real-Time PCR System (Applied Biosystems) and data was collected with ABI's Quantstudio 5 SDS Software. The experiments were conducted with PowerUp SYBR green PCR Master Mix (Applied Biosystems, USA), with a specific primer using the following PCR conditions: an activation stage at 95°C for 5 minutes and 40 cycles at 95°C for 15 seconds, 60°C for 30 seconds followed by melt curve at the following conditions: 95°C for 15 seconds, 60°C for 1 minute up to 95°C per second. The GAPDH gene was amplified in separate tubes as an active endogenous reference to normalize the quantification of a mRNA target. Fluorescence signal baseline and threshold were set manually for each detector generating a cycle threshold (Ct) for each sample. One sample of each group along with endogenous control was amplified with a no template control (NTC). The relative expression levels of the target genes were analysed by taking the base 2 logarithm transformed 2 $^{-\Delta Ct}$ values as a parameter. Results are represented as fold change values calculated using the 2 $^{-\Delta\Delta Ct}$ method. [30] Sample Control is taken as a control group and compared to all other groups for obtaining fold change i.e. RQ (relative quantitation) value.

RESULTS

1. **Teratogenesis-** Venlafaxine-treated embryos showed teratogenesis at 5mM and 10mM dose treatment. The treated embryos showed CNS defects like undifferentiated neuromeres, absence of brain divisions, and anophthalmia (Fig 1.2). The 5mM and 10mM Venlafaxine-treated embryos showed reduced body size, indistinct somites, and cervical flexion. Limb buds were well developed and elongated in treated embryos.

Figure 1: Venlafaxine-induced teratogenesis HH 24-25

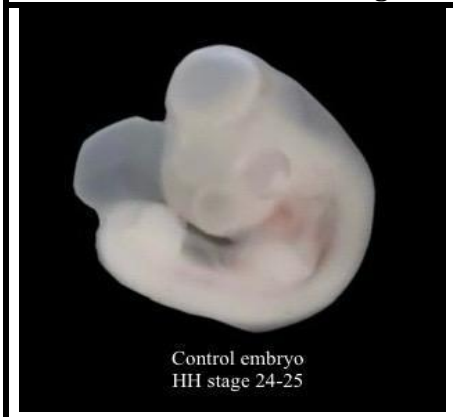


Figure 1.1

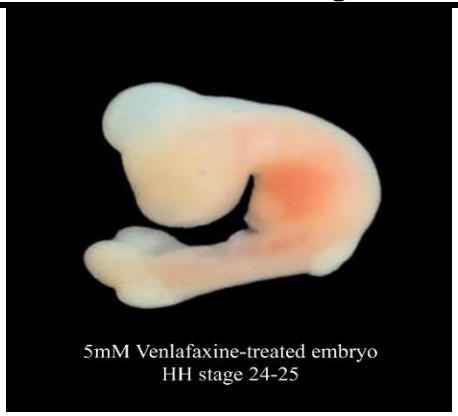


Figure 1.2

Figure 1.3

2. **Yolk Sac Membrane (YSM) Assay-** 5mM and 10mM Venlafaxine-treated embryos showed significant degeneration of YSM showing reduced number and thickness of blood vessels. Vessel diameter and density were reduced in treated embryos (Fig 2.2, 2.3) compared to control embryos (Fig 2.1). Venlafaxine treatment to the developing embryos induced degeneration of YSM, post 24 hours. YSM vessels were narrow, less turgid, and almost showed no presence of blood.

Figure 2: Angiogenesis (YSM Assay) for Venlafaxine-induced embryos HH 24-25

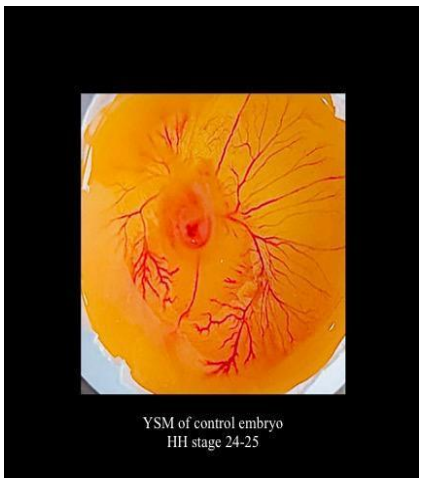


Figure 2.1

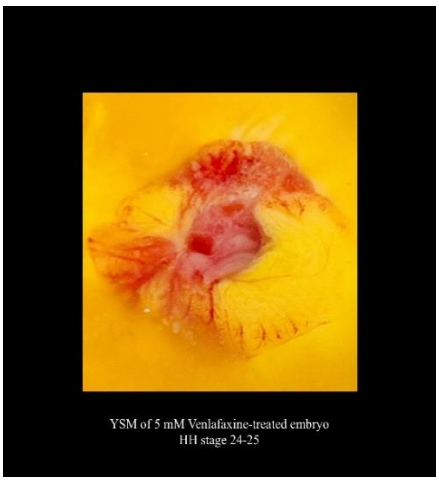


Figure 2.2

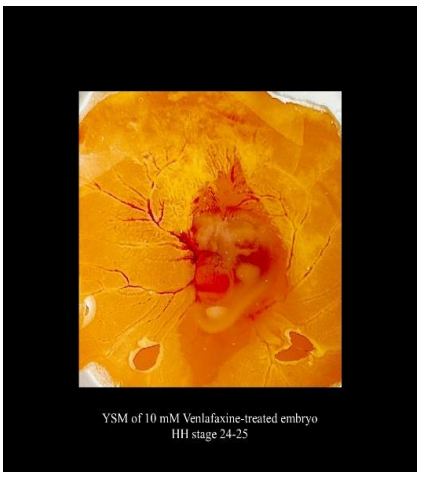


Figure 2.3

Figure 3: YSM Analysis

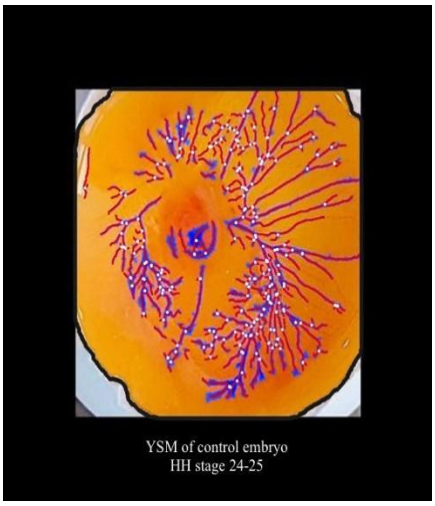


Figure 3.1

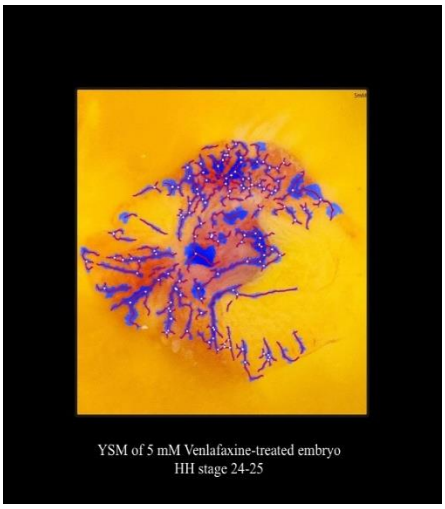


Figure 3.2

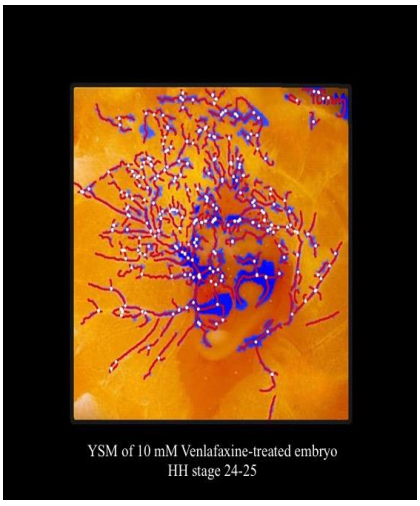


Figure 3.3

Figure 4: Graphical Analysis of YSM for Venlafaxine

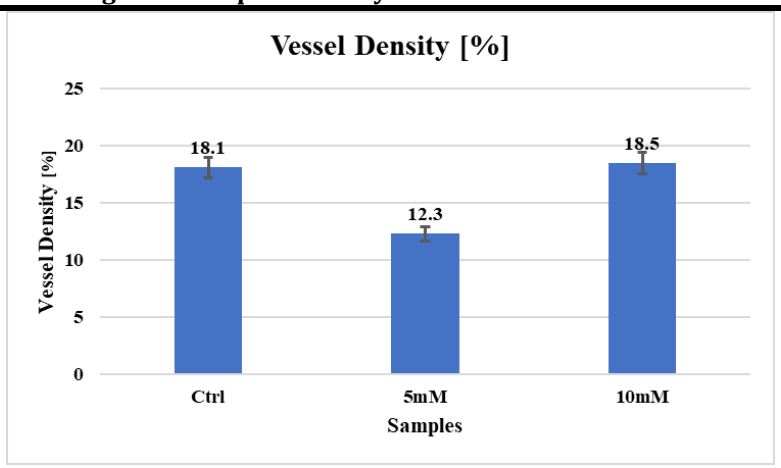


Figure 4.1

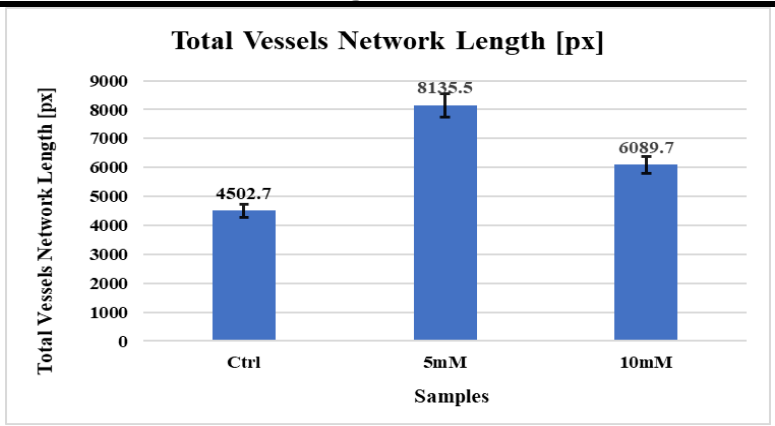


Figure 4.2

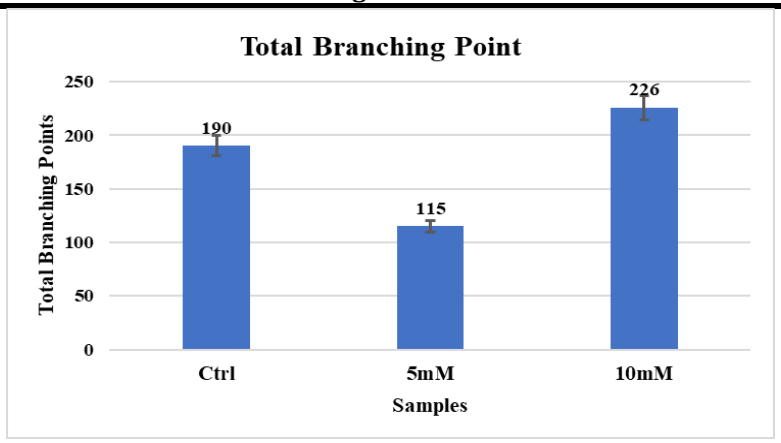


Figure 4.3

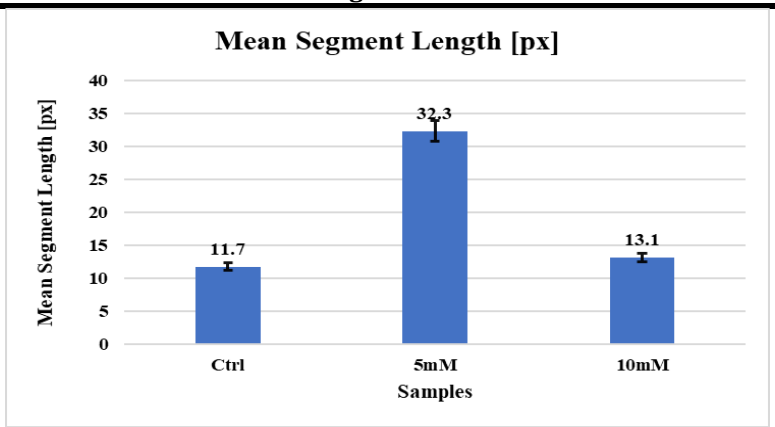


Figure 4.4

3. **Protein Biochemistry-** The results of the Bradford Assay indicated that embryos treated with 5mM Venlafaxine showed protein concentration of 174.19 µg/ml while the embryos treated with 10mM Venlafaxine showed 204.35 µg/ml, as compared to control embryos with 214.19 µg/ml protein concentration. SDS-PAGE analysis of the above protein samples displayed differentiation in gel bands. The molecular weights of the unknown gel bands were calculated using Standard Graph of Protein Ladder (Figure 9). [31] The protein samples of control embryos showed bands with molecular weights **120 kDa, 90 kDa, 79 kDa, 69 kDa, 63 kDa, 50kDa, 41kDa, 34 kDa, 30 kDa, 27 kDa, 20 kDa, and 19 kDa.** 5mM Venlafaxine-treated embryos showed comparatively fewer bands (**22 kDa, 20 kDa, 19 kDa**) out of which **20kDa** and **19kDa** correspond to control samples but **22kDa** was not observed in control. The gel band of 10mM dosage consisted of **120 kDa, 90 kDa, 69 kDa, 63 kDa, 41 kDa, 34 kDa, 30 kDa, and 19 kDa** values. Reported 10mM bands showed the absence of **79kDa, 50kDa, 27kDa, and 20kDa**, which were present in control embryo protein samples. Western Blotting analysis is required to confirm the identity of differentially expressed proteins in Venlafaxine-treated embryos.

Figure 5: Quantitative Analysis of Proteins using Bradford Assay

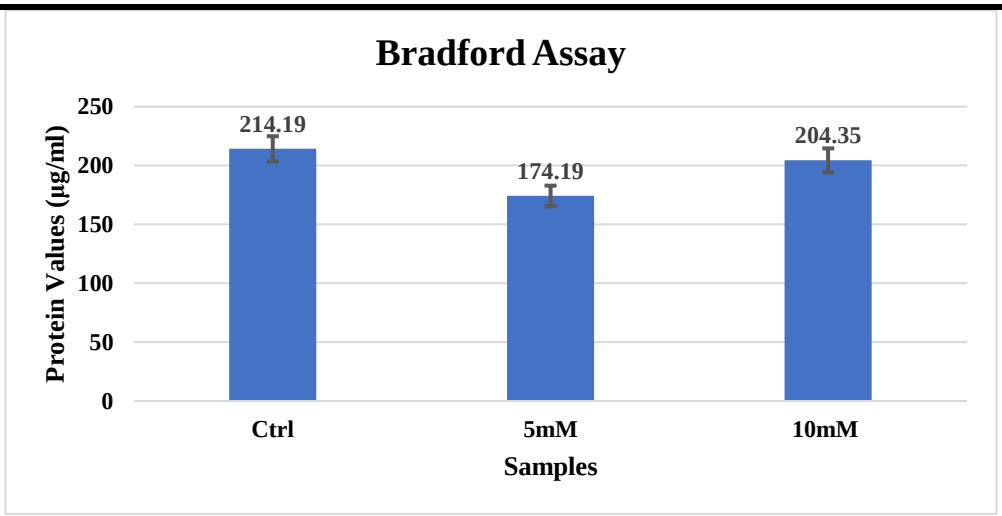


Figure 6: Qualitative Analysis of Proteins using SDS-PAGE

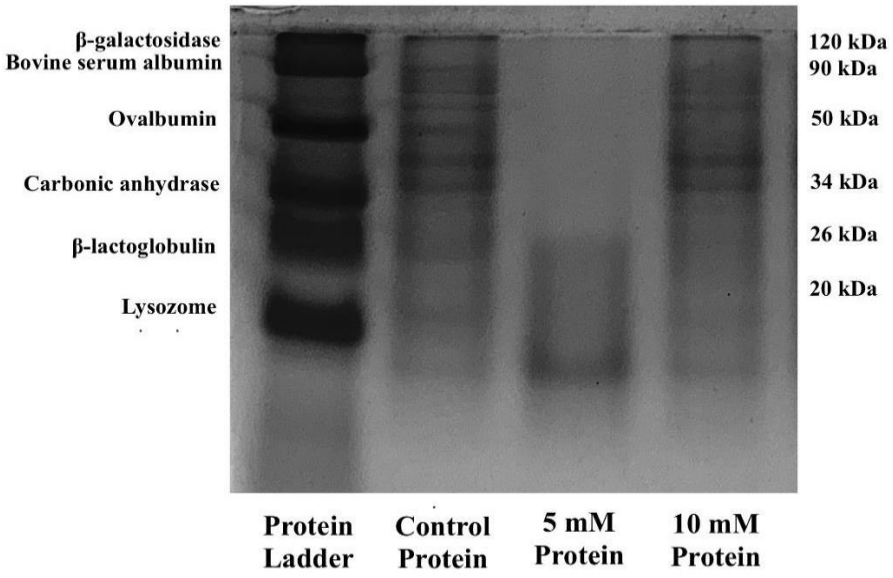


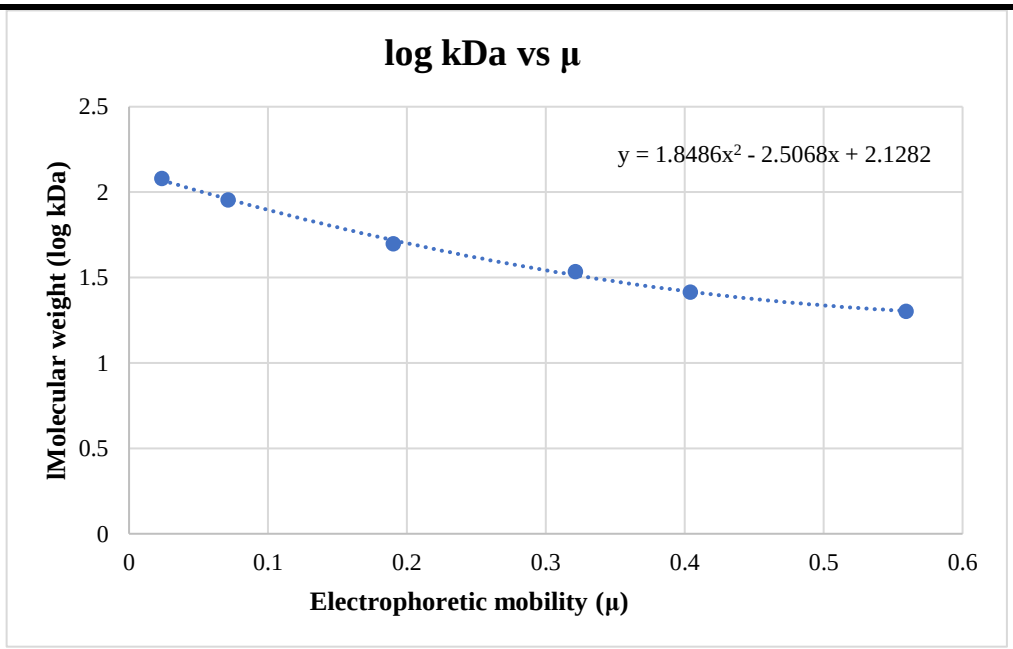
Figure 7: Band Profile of SDS-PAGE

Well No	Sample Name	Molecular Weights of Observed Protein Bands
1	Protein Ladder	**120 kDa, 90 kDa, 50 kDa, 34 kDa, 26 kDa, 20 kDa**
2	Control	**120 kDa, 90 kDa, 79 kDa, 69 kDa, 63 kDa, 50kDa, 41kDa, 34 kDa, 27 kDa, 20 kDa, 19 kDa**
3	5 mM Venlafaxine	**22 kDa, 20 kDa, 19 kDa**
4	10mM Venlafaxine	**120 kDa, 90 kDa, 69 kDa, 63 kDa, 41 kDa, 34 kDa, 30 kDa, 19 kDa**

Figure 8: Comparison of Band Profile

Mol. Wt (kDa)	120	90	79	69	63	50	41	34	30	26	22	20	19
Control	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	-	✓	✓
5mM	-	-	-	-	-	-	-	-	-	-	✓	✓	✓
10mM	✓	✓	-	✓	✓	-	✓	✓	✓	-	-	-	✓

Figure 9: Standard Graph of Protein Ladder



4. **Molecular Biology and Genomics** The fold change value for 10mM Venlafaxine-treated embryos was 2.272 and that of control embryos was found to be 1.000. The melting temperature for SHH is 87°C, and GAPDH is 86°C. Both control and Venlafaxine-treated embryos showed a Ct value of 20. Ct value for housekeeping gene GAPDH (internal control) was 18 for both control and Venlafaxine-treated embryos. Hence it can be seen that SHH gene expression was considerably increased in Venlafaxine-treated embryos.

Figure 10: qRT-PCR: Amplification plot for reference (housekeeping) gene: GAPDH

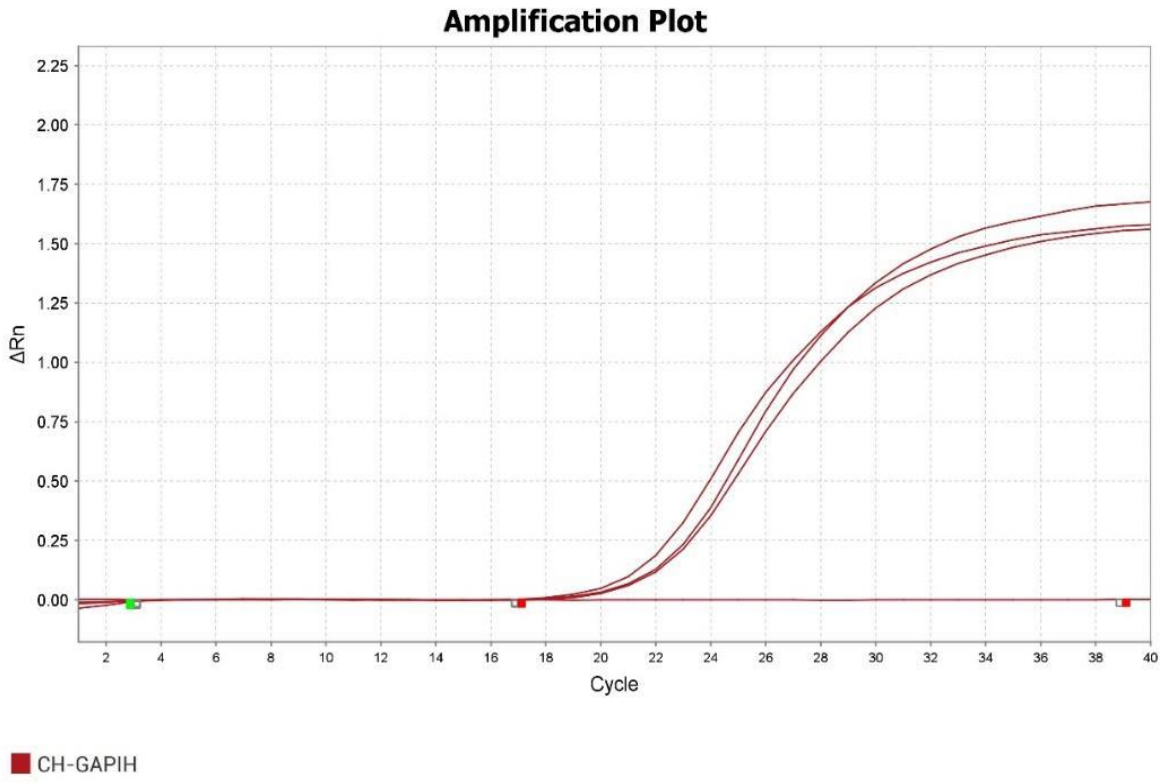


Figure 10.1

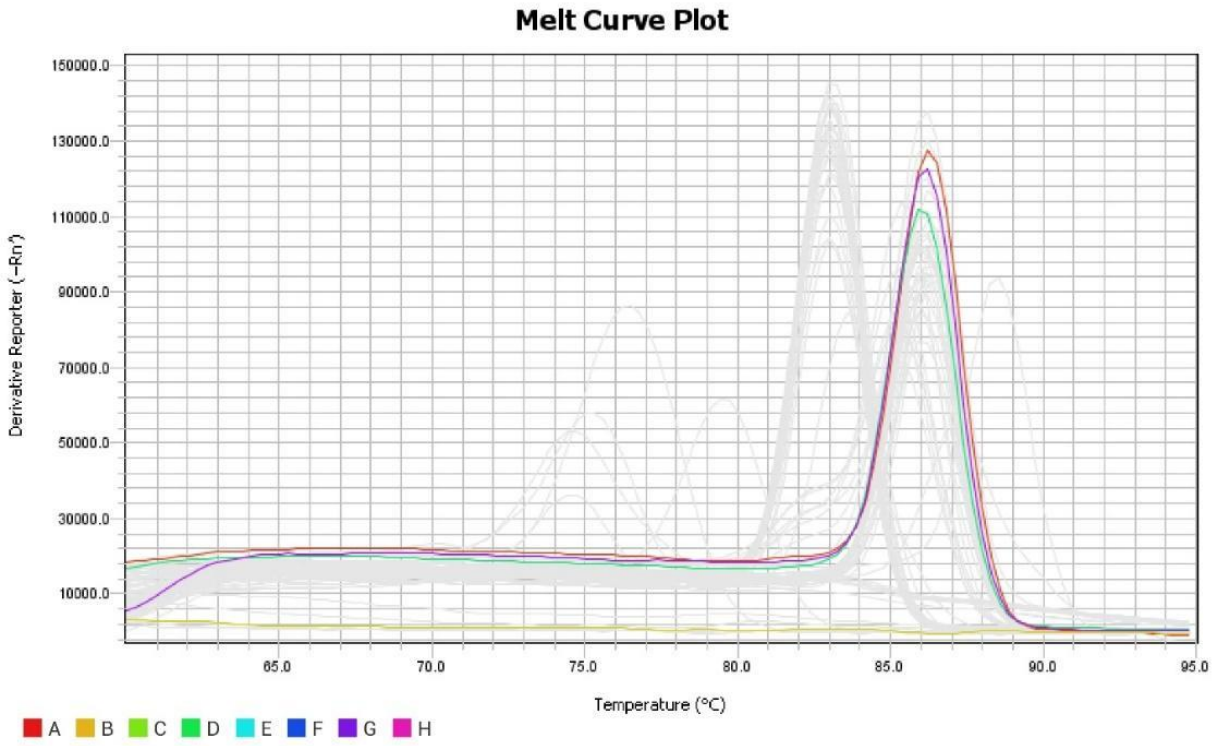


Figure 10.2

Figure 11: qRT-PCR: Amplification plot for target gene (SHH)

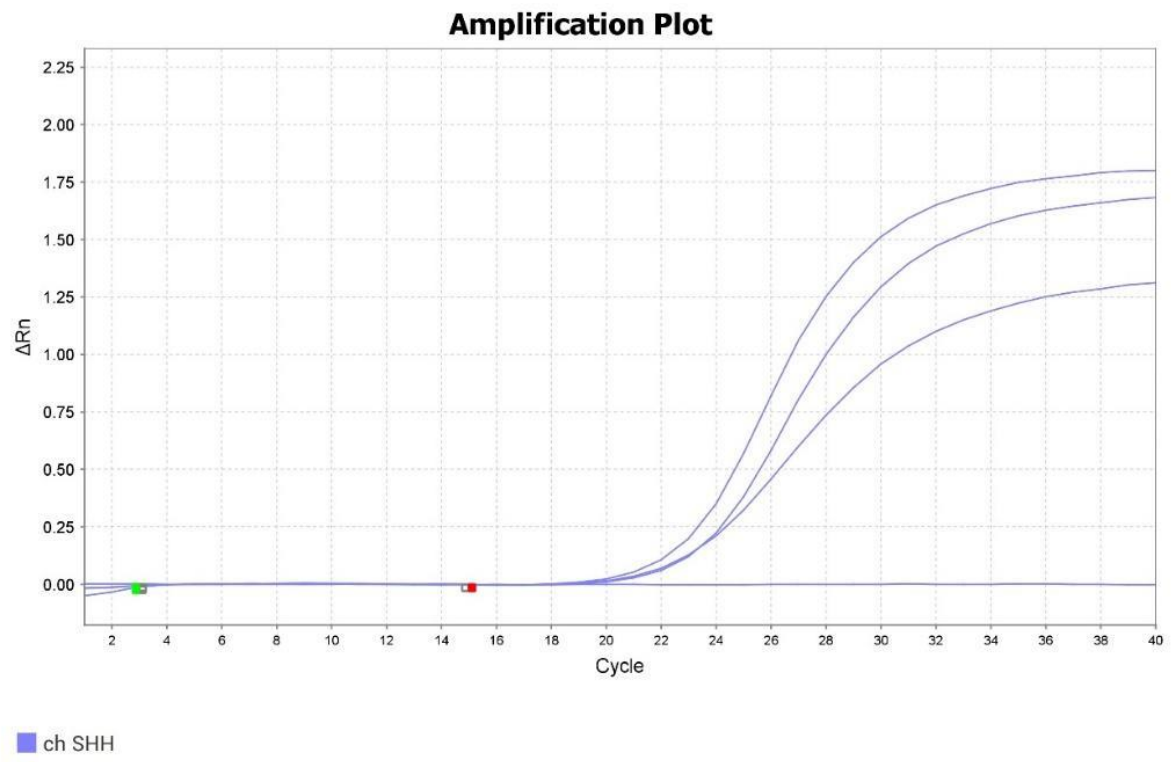


Figure 11.1

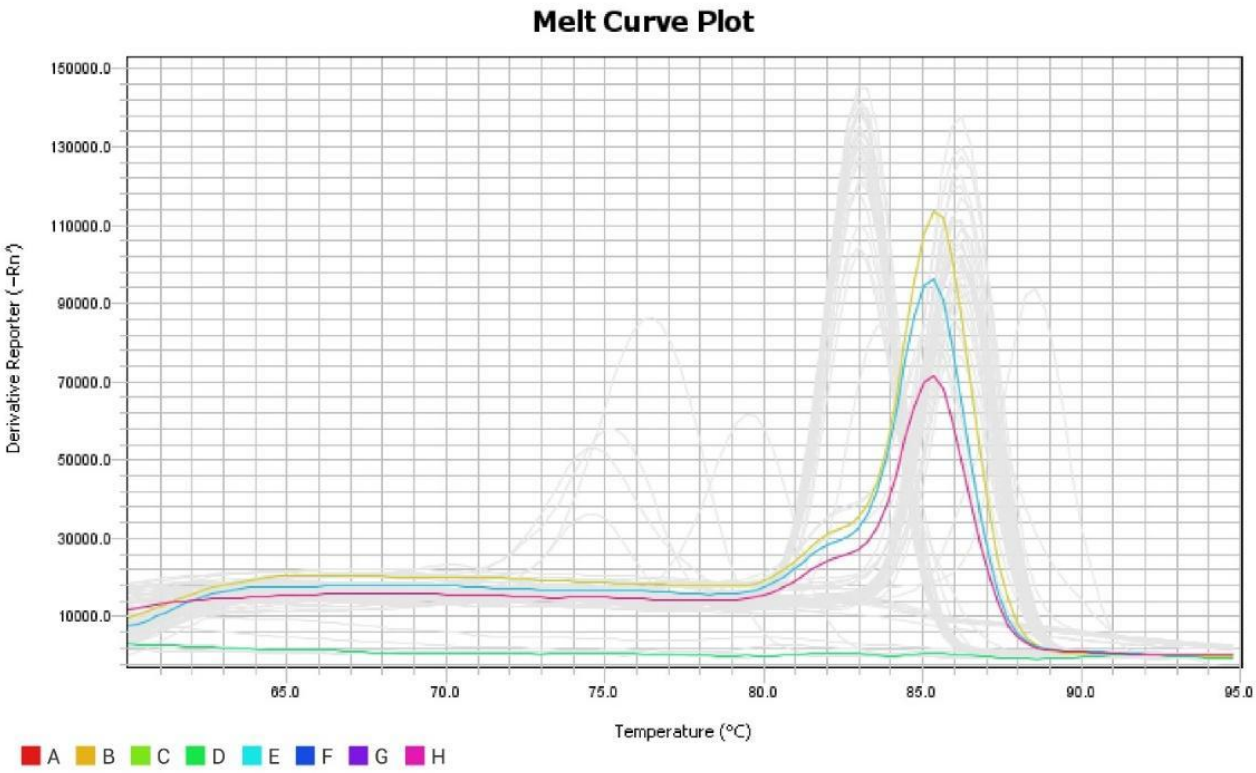
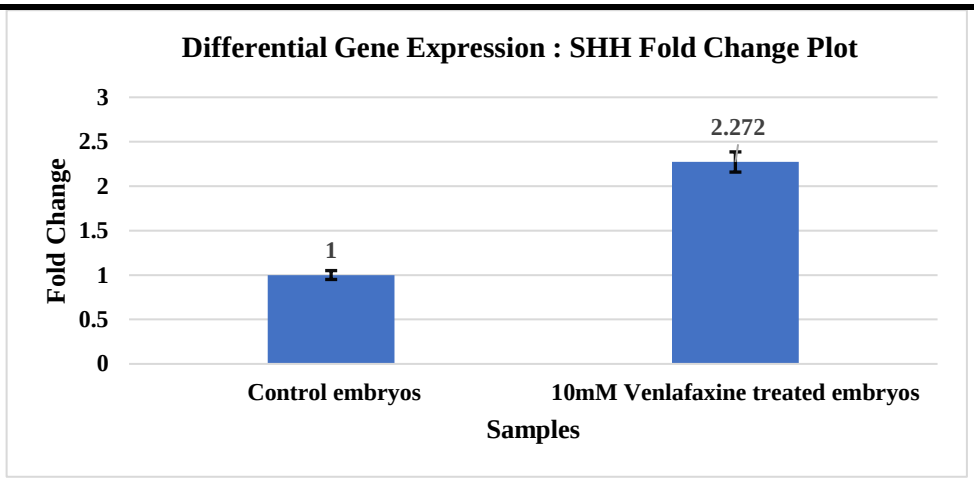


Figure 11.2

Figure 12: SHH gene expression: Relative Quantitation (fold change) value



These results provide insight into the developmental effects of Venlafaxine at the molecular level, warranting further investigation into its long-term consequences on embryonic development. PCR results demonstrated altered expression of the SHH gene in Venlafaxine-exposed embryos, suggesting that Venlafaxine interferes with this key signalling pathway, potentially leading to aberrant organogenesis and tissue differentiation.

DISCUSSION

Our studies reported embryotoxicity and teratogenicity of Venlafaxine at sublethal concentrations of 5mM and 10mM. Venlafaxine-treated embryos showed a considerable reduction in size when compared with the control embryos along with anophthalmia, hemorrhage, and reduction in blood vessel diameter of the Yolk sac membrane (YSM). Total degeneration of YSM vasculature was observed at a 10mM dose concentration, suggesting that SNRIs may interfere with angiogenesis and possibly hematopoiesis. Similar studies on Zebrafish using Venlafaxine were conducted which resulted in physiological changes like mortality, decreasing heartbeat, and premature hatching. [32,33] In the case of exposure of Venlafaxine to fathead minnows, there were no significant changes in the survival of the fish. [34]

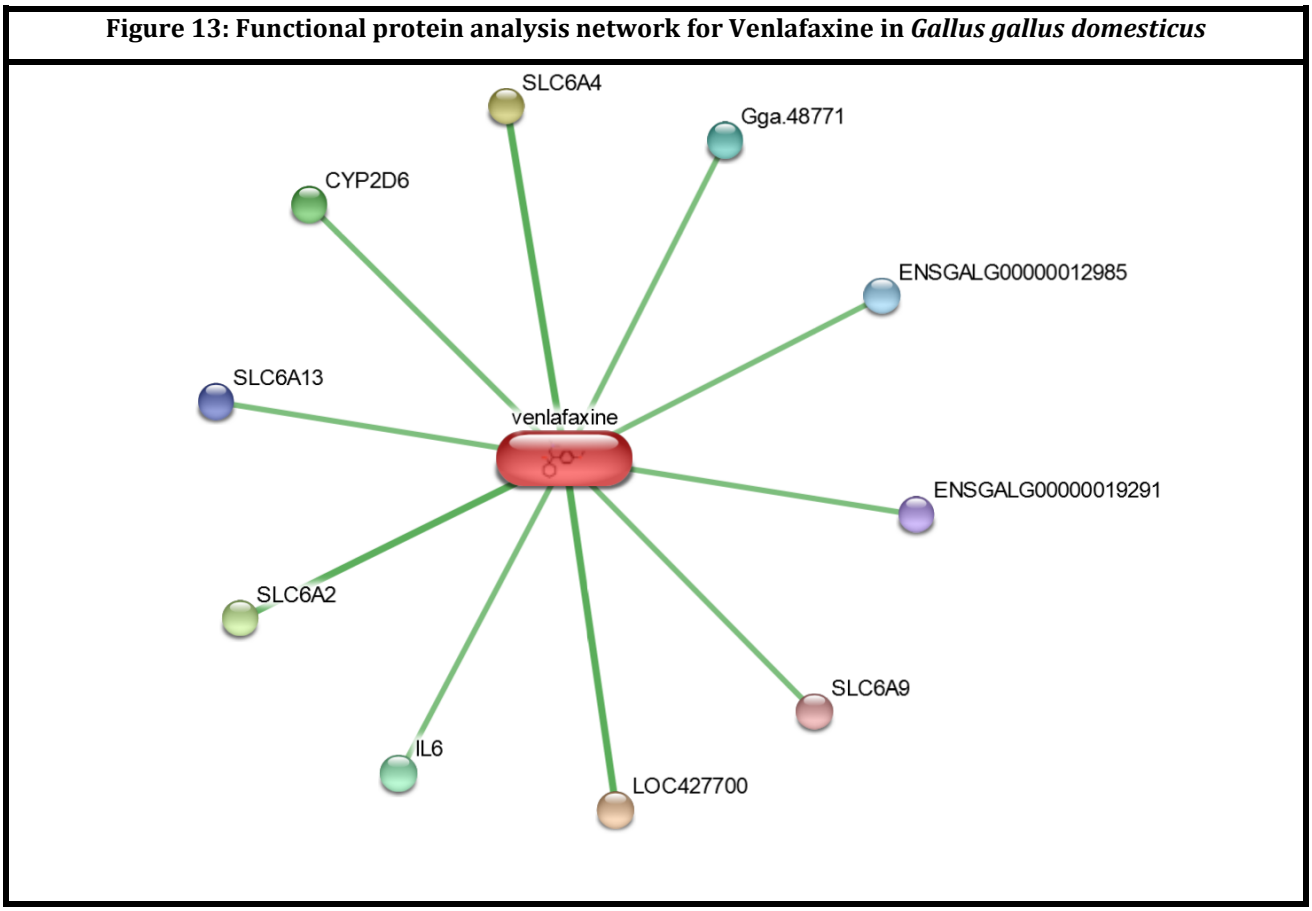
Total protein content was reduced in treated embryos (174.19 µg/ml, 204.35 µg/ml for 5mM and 10mM respectively) compared with the control embryos (214.19 µg/ml). This suggests that Venlafaxine exposure disrupts the normal pattern of protein expression in developing *Gallus gallus* embryos, potentially impacting essential pathways needed for proper growth and embryonic development. The specific changes observed in protein expression suggest that Venlafaxine may interfere with important signalling processes, increase metabolic stress, or affect structural proteins that play key roles in forming tissues and organs. These disruptions raise concerns about the possible effects of Venlafaxine on foetal development, highlighting the need for caution when considering antidepressant use during pregnancy.

Teratogenic studies, YSM assay, and SDS-PAGE analysis showed that 5mM Venlafaxine-treated embryos exhibited anomalous results. YSM analysis showed that 5mM Venlafaxine-treated embryos showed lesser vessel density % and lesser total branching points as compared to control and 10mM treated embryos. In contrast, mean segment length [px] and total vessel length [px] exhibited an increase in 5mM Venlafaxine-treated embryos. This may be possible due to stage-specific targeting of proteins by Venlafaxine (upregulation and downregulation of developmentally regulated genes). This points to the fact that Venlafaxine has a profound effect on angiogenesis and vasculogenesis in the developing embryo. Research conducted on Zebrafish embryos focusing on the treatment of Venlafaxine, Carbamazepine, and Tramadol exhibited oedema and distortion of the yolk sac, along with blood circulation anomalies. [35]

SDS-PAGE analysis of 5mM Venlafaxine-treated embryos showed a decrease in protein bands, as only bands having **19 kDa, 20 kDa,** and **22 kDa** molecular weights were observed. In the protein samples of embryos treated with 10mM Venlafaxine, the band profile showed molecular weights of **120 kDa, 90 kDa, 69 kDa, 63 kDa, 41 kDa, 34 kDa, 30 kDa,** and **19 kDa**. From the reported protein bands containing the above molecular weights, **22 kDa** from 5mM Venlafaxine-treated protein sample and **30 kDa** from 10mM Venlafaxine-treated protein sample were not present in control embryos. Previous studies of Venlafaxine activity by Enzyme-linked immunosorbent assay (ELISA) conducted on Wistar rat cell culture exhibited a decrease in mean IL-6 concentration when treated with 30ng/ml Venlafaxine, but the same effect was not seen at a higher concentration of 300ng/ml. [36] A similar observation was recorded in our studies of protein biochemistry, where low concentration (5mM) of Venlafaxine showed lower protein content (174.1 µg/ml) as compared to control embryos (214.19 µg/ml) and embryos treated with 10mM concentrations of the drug (204.35 µg/ml).

Functional protein analysis network showed that the following proteins in the developing stages of *Gallus gallus domesticus* are affected by Venlafaxine:

Figure 13: Functional protein analysis network for Venlafaxine in *Gallus gallus domesticus*



(Source: STITCH Database) [37]

IL-6 is a cytokine, while LOC427700, Gga.48771, ENSGALG00000012985, ENSGALG00000019291, SLC6A13, are transporters. SLC6A4 is a sodium-dependent serotonin transporter, SLC6A2 is a sodium-dependent noradrenaline transporter, SLC6A9 is a sodium and chloride-dependent glycine transporter 1 and CYP2D6 is a cytochrome P450, family 2, subfamily D, polypeptide 6.

Venlafaxine potentially has a reciprocal influence on pathogenic genes such as BDNF, HTR1A, SLC6A3, and SLC6A4. It is chiefly metabolized by CYP2D6 and CYP3A4 enzymes with minor metabolism by CYP2C9 and CYP2C19. Also, it has weak inhibitory effects on genes like CYP2B6, CYP2D6, SLC6A2, SLC6A3, and SLC6A4. [38] Studies found that remission with Venlafaxine–XR was associated with the CYP2D6 metabolism phenotype. More specifically, the rate of remission was statistically higher amongst Ultra-Rapid Metabolizers URM (n = 5, 71.4%) than among Poor Metabolizers PM (n = 1, 10%). It was hypothesized that a linear relationship existed between CYP2D6 metabolizer status and the odds of Venlafaxine-XR remission, from PM to Intermediate Metabolizers/ Extensive Metabolizers and finally to URM. The findings revealed that the odds of remission increased with increasing metabolic rate. [39] In studies conducted for individuals with low expressing genotypes ($1_A1_G/1_G1_G/s1_A/s1_G/ss$) and high expressing genotype (1_A1_A) of SLC6A4, the patients with high expressing genotypes showed a higher percentage of poor responses when treated with Venlafaxine as compared to patients carrying the low expressing genotype. [40] In another research where high analgesia (HA) mice were treated with Desipramine, results showed an upregulation of genes involved in neurotransmitter transport and termination of GABA and glycine activating (SLC6A11, SLC6A9) and glutamate (SLC17A6). [41] Further confirmation of these protein bands needs to be done by Western Blotting.

The qRT-PCR analysis revealed that the target gene (Sonic Hedgehog) was overexpressed by approximately 2.727 times in the 10mM Venlafaxine-treated embryos as compared to control embryos, which had a value of 1.0. This suggests a possible upregulation of SHH in dose-response to Venlafaxine. Sonic hedgehog protein facilitates cell growth, normal spacing of the body, and cell specialization. Due to chemical signalling by the SHH gene, differentiation between the left and right hemispheres of the forebrain is seen. Sonic hedgehog protein also causes the separation of two different eyes from the eye field during early eye formation. SHH determines the anteroposterior axis in the limb. SHH gene expression is restricted posteriorly throughout limb development, and SHH protein makes a density gradient from the posterior to the anterior side, specifying digits and identities. [42] Previous studies have shown that activation of the Gli1 gene due to SHH signalling promotes the antidepressant effect observed in gestating women. [43] There is an observable dose-dependent decrease in BDNF (brain-derived neurotrophic factor) expression, as opposed to an increase in 5HTR1A expression, in 200μM Venlafaxine-treated cells. [44] Venlafaxine causes concentration-dependent up-regulation of the BDNF promoter IV in differentiating SH-SY5Y neurons. It negatively affects

neurite growth within 48 hours, with 10μM concentrations being the most significant. Venlafaxine, an SNRI, has neurodevelopmental effects when presented to developing neurons, through an increase of 5-HT and norepinephrine. [45]

CONCLUSION

SNRIs are used against Major Depressive Disorders which show symptoms like obsessive-compulsive disorder, anxiety, and panic disorder. Administration of SNRIs may also influence the serotonergic system as both these neurotransmitter systems are interlinked. Hence, they should be used cautiously as they may cause embryotoxicity and teratogenesis in developing embryos.

These results provide insight into the developmental effects of Venlafaxine at the molecular level, warranting further investigation into its long-term consequences on embryonic development. qRT-PCR results demonstrated altered expression of the SHH gene in Venlafaxine-exposed embryos, suggesting that Venlafaxine may interfere with the SHH signalling pathway, potentially leading to aberrant organogenesis and tissue differentiation, leading to teratogenesis. However further studies are required on the same, leading to the development of safer and more potent non-teratogenic antipsychotics.

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