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Research Article

SNRI (Venlafaxine) induces Neural Tube Defects (NTDs) in Developing Chick Embryos

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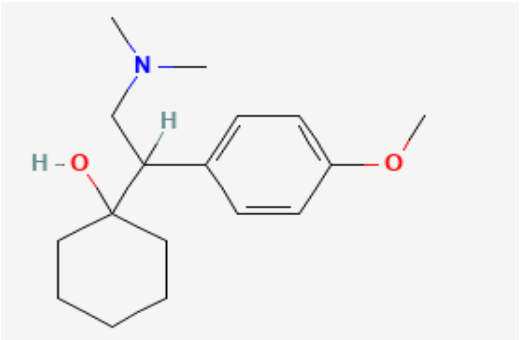
Abstract

This research investigates the teratogenicity of SNRI (Venlafaxine) on neural tube formation in *Gallus gallus domesticus* (chick embryos). Fertilized eggs were exposed to two levels of Venlafaxine (500 ppm and 1000 ppm), and embryonic results were evaluated after 24 hours of incubation. Morphological screening detected evidence of neural tube defects (NTDs) in treated embryos, such as wavy neural tube. These phenotypic defects were associated with dose-dependent decreases in major biochemical markers: total protein (control-1.7 Gms%, 500 ppm-1.38 Gms%, 1000 ppm- 1.4 Gms%), albumin (control- 1 Gms%, 500 ppm- 0.76 Gms%, 1000 ppm- 0.88 Gms%), globulin (control- 0.7 Gms%, 500 ppm- 0.62 Gms%, 1000 ppm- 0.52 Gms%) and alkaline phosphatase (ALP) levels in treated embryos versus controls. Given the key role of alkaline phosphatase in early neurodevelopment and tissue differentiation, its inhibition could play a role in defective neural tube closure. These observations indicate that Venlafaxine interferes with early embryogenesis, specifically with neural tube formation, and raise concerns regarding its safety in pregnancy and emphasize the importance of rigorous risk assessment for prenatal use of antidepressants.

Keywords: Venlafaxine, Teratogenesis, Chick embryo, SNRIs (Serotonin-Norepinephrine Reuptake Inhibitor), Antidepressant, Total protein, Serum Albumin, Serum Globulin, Alkaline Phosphatase (ALP), SDS-PAGE, Neural Tube Defects (NTDs)

INTRODUCTION

Venlafaxine is a type of antidepressant that belongs to the class known as the Serotonin-Norepinephrine Reuptake (SNRIs). It has a base structure of N, N-dimethylethanamine which is substituted by a 1-hydroxycyclohexyl and 4-methoxyphenyl group at position 1 [1].



https://pubchem.ncbi.nlm.nih.gov/compound/Venlafaxine

Figure 1: Structure of Venlafaxine

It blocks the transport proteins which increases the serotonin levels, which results in the stoppage of their reuptake at the presynaptic terminal. This increases the postsynaptic stimulation as more transmitters are available in the synapse [2]. SNRIs' primary target is serotonergic and noradrenergic neurons but have almost no effect on cholinergic or histaminergic receptors. Venlafaxine is more of a serotonin reuptake inhibitor than norepinephrine reuptake. It was marketed as Effexor. It can be used to treat major depressive disorder, generalized anxiety disorder, panic disorder and social anxiety disorder approved by FDA. Venlafaxine tablet is prescribed for long-term treatment to be taken orally, and has serious side-effects if not taken as per the prescription or stopped abruptly. Sudden stoppage of the dosage can have serious side-effects such as irritability, tiredness, restlessness, anxiety, insomnia, trouble sleeping, nightmares, headache, sweating, dizziness, tingling, or "pins and needles" feeling, shaking, confusion, nausea, vomiting, or diarrhoea [3]. Symptoms of an overdose of Venlafaxine can include: tachycardia, unusual sleepiness, dilated pupils, seizures, vomiting, cardiac arrhythmias, hypotension, muscle aches or pains, or dizziness. Venlafaxine has severe toxicity effects especially when combined with other antidepressants such as SSRIs (Fluoxetine), SNRI or MAOI, which can lead

to serotonin syndrome. Serotonergic activity rises to life-threatening levels in the Central Nervous System (CNS) [2]. This can lead to the diagnosis of hyperactivity, mental status changes, and neuromuscular abnormalities. Serotonin syndrome characteristically presents with myoclonus, agitation, abdominal cramping, hyperpyrexia, hypertension, and potentially death. No lab test exists to confirm the diagnosis of serotonin concentrations as they do not correlate with the symptoms clinically. Hunter Toxicity Criteria Decision Rules can be used to form the diagnosis [4]. Bird models like *Gallus gallus domesticus* (domestic chicken) are significant models because of their close resemblance to humans [5]. They are at an advantage for studying transgenerational epigenetic inheritance. As the interval between two generations is short, they show early sexual maturity, high egg production rate. All these factors make them valuable for assessing long term effects of pharmaceutical exposure [6]. Venlafaxine increased the expression of *MDR* gene in human as well as mice cells while simultaneously suppressing p-glycoprotein levels in human cells. In mice this suppression of p-glycoprotein inhibits dendritic cells development [7,8]. Further studies in mice assessed the genotoxicity and cytotoxicity profile of Venlafaxine. These toxicities were prominent with the highest dosage used i.e 250mg/kg, in the acute assay. On the other hand, in the subchronic assay genotoxicity was observed in the last three weeks with the highest dose evaluated i.e 10mg/kg [9]. Additionally, Venlafaxine has dose-dependent on human genetic material specifically by increasing Sister Chromatid Exchange (SCE) frequency and inhibiting Proliferation Rate Index (PRI) and Mitotic Index (MI) levels in human lymphocytes [9]. When paired with Chronic Mild Stress (CMS), Venlafaxine showed an increased down-regulation of hippocampal *CAT* and *SOD1* expression. Along with this there was an increase in the expression of *Gpx1* in the midbrain followed by increased expression of *Gpx4* and *NOS1* in the midbrain as well as the nuclear basal ganglia. As a result, these returned back to control levels after the application of Venlafaxine [11].

MATERIALS AND METHODS

A) Teratogenesis

Chick embryo culture and treatment with Venlafaxine

Fertilized chicken eggs (*Gallus gallus*), white Leghorn strains were acquired from Venkateshwara Hatcheries, Pune Pvt. Ltd. After eggs were obtained, they were cleaned using distilled water and 70% ethanol then placed in a BOD incubator for 24 hours prior to treatment. Relative humidity (Rh) was maintained at 70-80% and temperature set at 37.8°C. After the incubation, these eggs were opened with window slit method from the vegetal pole. The embryo was made visible by discarding the excessive albumen. The embryo was exposed by cutting the vitelline membrane. The embryos were treated with 100µL of 500ppm and 1000ppm Venlafaxine solution respectively along with a control embryo. The embryos were further kept for incubation in the same conditions as mentioned above for 24 hours. After 24 hours of incubation the control and treated embryos were screened for teratogenesis which is discussed in the Results.

B) Biochemical Studies

Protein extraction and estimation

Control and treated embryos were homogenized in 1X Protein Extraction Buffer (PEB) (50mM Tris, pH 7.4, 250mM NaCl, 5mM EDTA, 50mM NaF, 1 mM Na_3VO_4, 1% NP-40, 0.02% NaN_3) in a Potter Elvejhem homogenizer on ice. The homogenates were centrifuged at 4°C at 3000rpm for 5 minutes. The supernatant was transferred to another clean sterile Eppendorf tube and stored at -20°C for further use. Bradford assay was used for protein estimation [12]. Qualitative analysis of proteins from control and Venlafaxine treated chick embryos was performed using SDS PAGE. Band intensities were calculated using ImageJ software® (https://imagej.net/ij).

RESULTS

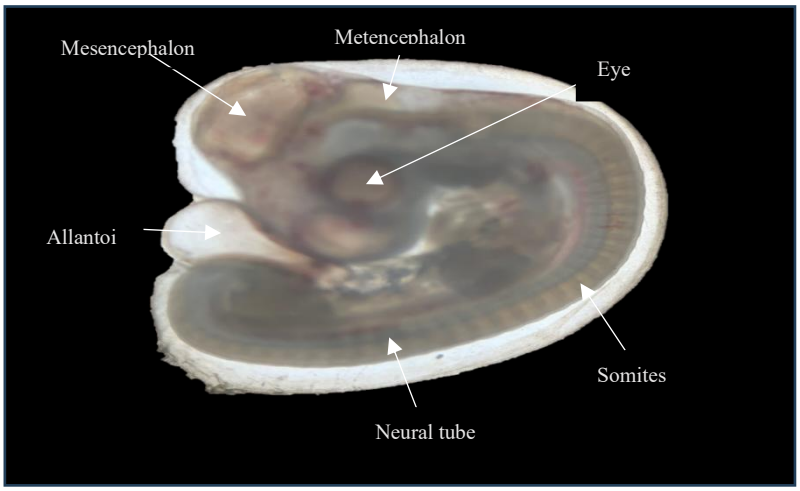


Figure 2.1: Control chick embryo

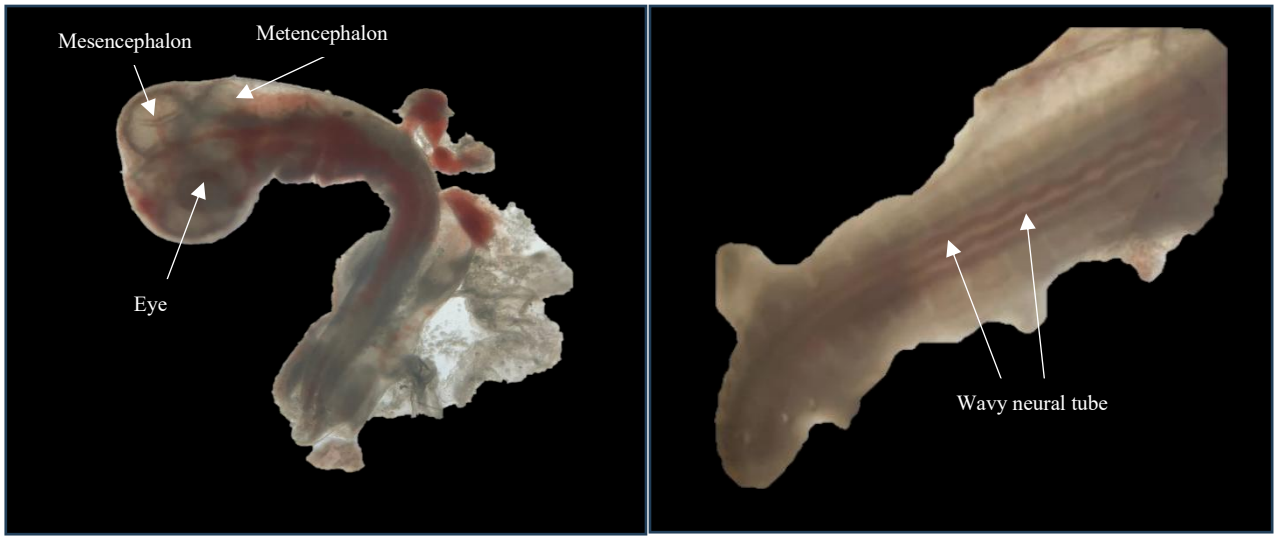


Figure 2.2: Chick embryo treated with 500ppm Venlafaxine

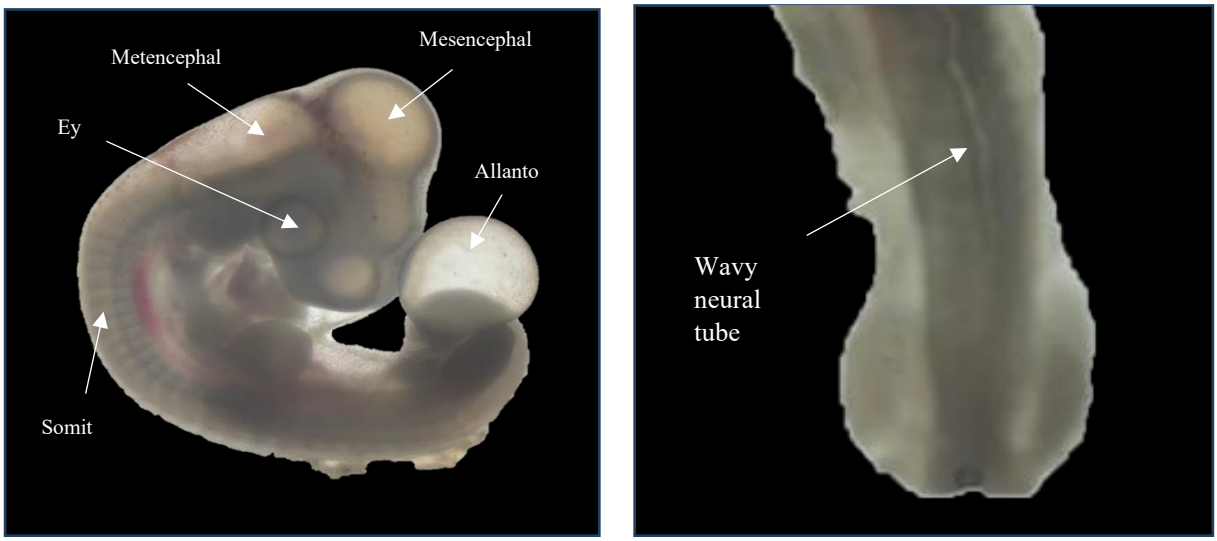
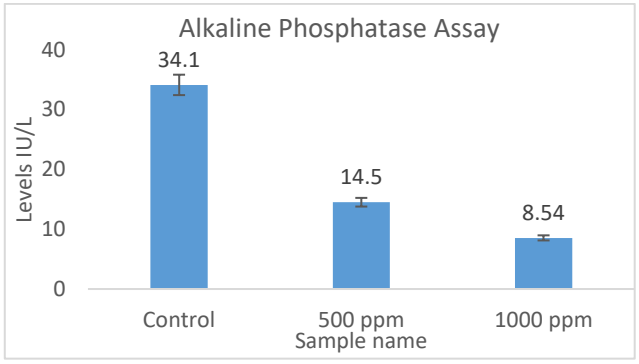


Figure 2.3: Chick embryo treated with 1000ppm Venlafaxine

Biochemical Studies

Enzyme Assays: Alkaline Phosphatase (ALP)

Effect of Venlafaxine of ALP

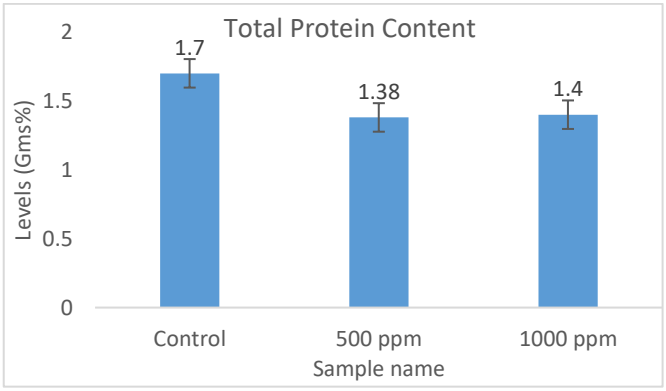


Graph 1.A

Quantitative analysis of Alkaline Phosphatase showed that activity decreased in a dose-dependent pattern. Embryos treated with 500ppm showed ALP activity **14.5 IU/L**. At higher dose of 1000ppm the ALP activity was **8.54 IU/L**. The control embryo showed ALP levels **34.1 IU/L**.

TOTAL PROTEIN

EFFECT OF VENLAFAXINE ON TOTAL PROTEIN CONTENT

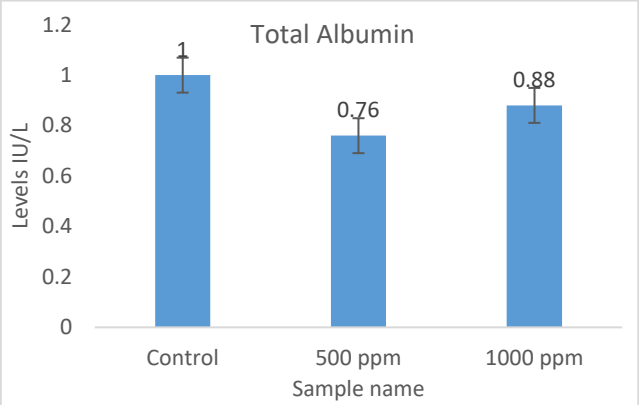


Graph 1.B

There was also a decrease in total protein levels, although it was not dose-dependent. Control embryo showed total protein value of **1.7 Gms%** which decreased to **1.38 Gms%** and **1.4 Gms%** upon treatment by 500ppm and 1000ppm doses, respectively.

TOTAL ALBUMIN

EFFECT OF VENLAFAXINE ON TOTAL ALBUMIN

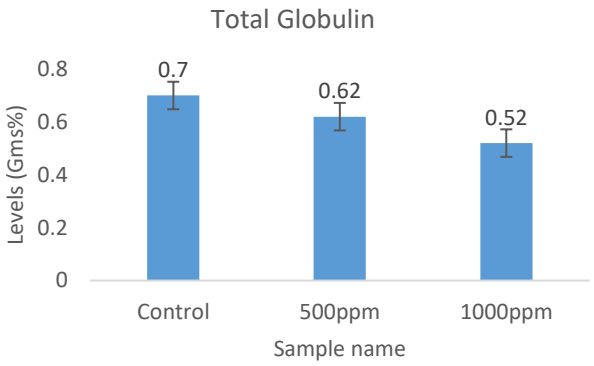


Graph 1.C

A similar dose-independent decrease was observed in total albumin. **1 Gms%** total albumin was observed in control embryos. On the other hand, this value decreased to **0.76 Gms%** and **0.88 Gms%** in 500ppm and 1000ppm treated embryos, respectively.

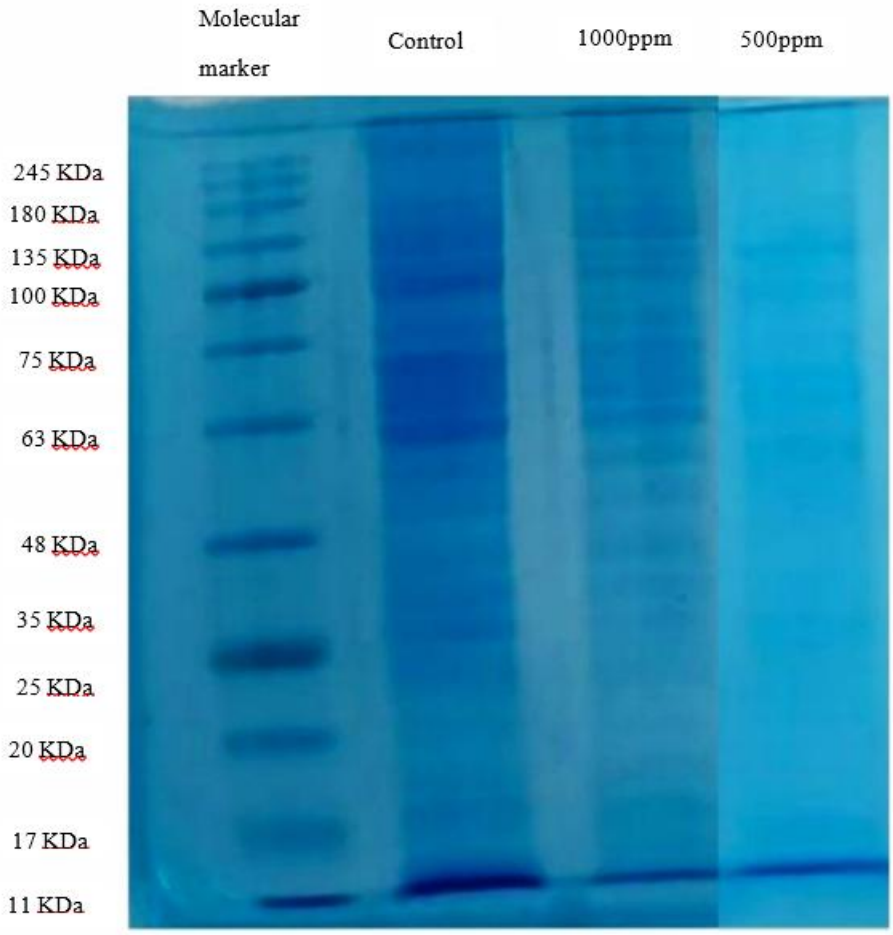
SERUM GLOBULIN

EFFECT OF VENLAFAXINE ON TOTAL GLOBULIN



Graph 1.D

The decrease in total globulin was dose-dependent, from **0.7 Gms%** in control embryos to **0.62 Gms%** in 500ppm Venlafaxine treated embryos and **0.52 Gms%** in 1000ppm Venlafaxine treated embryos.



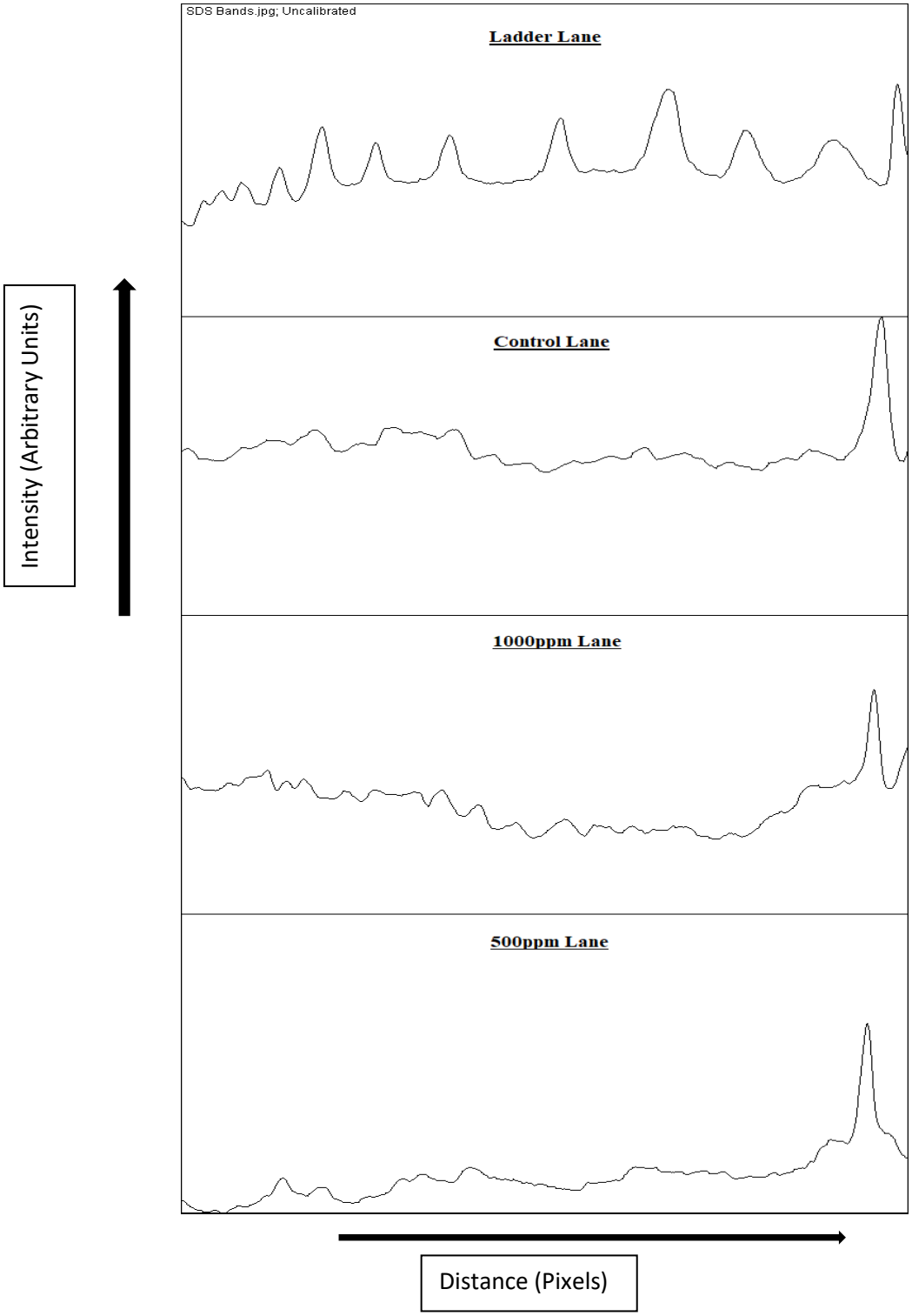
SDS-PAGE

Fig 3.1

The following plots are SDS-PAGE plots obtained from analysis using ImageJ software® (https://imagej.net/ij). Each peak corresponds to a single band in the respective lanes. The area under each peak equates to the intensity of the band.

SDS PAGE Plots

Fig 3.2



SDS-PAGE Band Intensities

Table 2.1

Lane 1	Bands	Intensity	Lane 2	Bands	Intensity	
Molecular	1	46.607	Control	1	36.192	
weight	2	64.485	embryo	2	23.364	
marker	3	232.971		3	297.092	
	4	449.263		4	346.698	
	5	1003.335		5	172.435	
	6	521.385		6	257.263	
	7	564.385		7	3186.184	
	8	833.213				
	9	2051.941				
	10	1133.82				
	11	1508.426				

Table 2.2

Lane 3	Bands	Intensity	Lane 4	Bands	Intensity	
1000ppm	1	25.657	500ppm	1	227.556	
Venlafaxine	2	98.556	Venlafaxine	2	175.021	
	3	73.192		3	71.728	
	4	140.799		4	71.728	
	5	115.435		5	63.071	
	6	62.778		6	248.971	
	7	53.485		7	1576.335	
	8	342.335				
	9	235.506				
	10	82.263				
	11	416.749				
	12	25.485				
	13	60.899				
	14	19.364				
	15	1310.456				

The above tables show band intensities of molecular ladder and control embryo (Table 2.1), and 500ppm Venlafaxine treated embryo and 1000ppm Venlafaxine treated embryo (Table 2.2). The intensities are calculated using arbitrary units, reflecting relative intensity values. Combining these with figures 3.1 and 3.2 we see a disruption in the protein expression profiles of chick embryos treated with Venlafaxine.

DISCUSSION

Our studies in the chick embryo system complemented teratogenic findings in the existing literature by providing evidence of the teratogenic potential of Venlafaxine. Treated embryos specifically showcased morphological abnormalities in the form of neural tube defects (NTDs). A wavy neural tube was observed, which was indicative of impaired neural tube closure. These teratogenic effects were accompanied by biochemical disruptions in levels of total protein, albumin, and globulin. The most notable decrease in the enzyme levels was for alkaline phosphatase (ALP) activity in a dose-dependent pattern, from **34.1 IU/L** in control embryos to **14.5 IU/L** and **8.54 IU/L** at 500 ppm and 1000 ppm, respectively.

The current literature shows that Venlafaxine use is associated with high risk of congenital defects in mice [13]. Studies involving exposure of Venlafaxine to rats resulted in the experimental animals experiencing seizure, coma, and death because of the harmful effects of Venlafaxine on liver, kidney, and stomach at a single high dose or repeated exposure to ten times the therapeutic doses. In human clinical settings Venlafaxine has been implicated in causing adverse cardiovascular side-effects such as angina pectoris, hypotension, dose-dependent elevated levels of BP, syncope, sinus bradycardia and first-degree of arterioventricular block (AV) bundle branch block. Acute heart failure was also

observed in the patients with high dose [14]. A 41-year-old female developed major central nervous system depression upon ingestion of 4.5 g venlafaxine, 50 mg diphenhydramine, 50 mg thiothixene. She also showed elevated systolic and diastolic blood pressures and sinus tachycardia [15]. As evidenced by the case of a 29-year-old male, Venlafaxine overdose may result in cardiovascular and neural toxicity [16]. Other studies have reported Venlafaxine's effects beyond embryogenesis, such as inducing apoptosis in melanoma cells by activating JNK ½ induced Nur77 expression, triggering mitochondrial localized apoptosis [11]. Cetrizine Venlafaxine, O-desmethylvenlafaxine, and metformin were found in human breast milk and plasma [17]. Additionally, serotonin-norepinephrin reuptake inhibitors (SNRIs) treatment has been linked to malformations of the nervous system and congenital cardiovascular defects [18]. In zebrafish Venlafaxine has shown to stimulate neurogenesis and disrupt larval behaviour. Thus, Venlafaxine might hinder zebrafish development [19].

ALP has an important role in early neurodevelopment, especially in tissue differentiation and neural tube formation. The tissue non-specific ALP isoforms are found to be highly expressed in the developing nervous system and are associated with neurogenic activity [20]. Therefore, an inhibition in ALP activity, such as that observed in our study, may contribute to the treatment-induced Neural Tube Defects (NTDs) in the embryos. Venlafaxine's known interaction with genes such as CYP2D6, SLC6A2, SLC6A9, and IL6 in *Gallus gallus domesticus* [21] may further influence neurodevelopmental gene expression and inflammatory responses, which are both implicated in embryonic malformations. Research shows that CYP2D6 phenotype in an individual may be a probable parameter for Venlafaxine induced toxicity [21]. CYP2D6 catalyses the oxidation pathway of Venlafaxine, further providing a link between the two. Although previous clinical studies have reported elevated ALP levels in adult patients treated with Venlafaxine—presumably hepatic in origin—our findings in embryos suggest tissue- and developmental-stage-specific enzymatic suppression [23]. Venlafaxine has shown an effect of disrupting protein expression. The NCCIT cell derived Embryoid Bodies (EBs) were treated with Venlafaxine and this reduced the expression of HIP2 and T-plastin. On the other hand, the levels of PKM and TGF-β3 were increased after the first day of testing. On further testing after 7 days, P4HB and T-plastin mRNA levels were decreased further and the expression of PKM and DPYSL3 were increased. Also, the levels of HIP2 after day 7 decreased [24]. These results are compatible with our findings of protein expression disruption in Venlafaxine treated chick embryos.

CONCLUSION

This research shows that Venlafaxine exposure has an adverse, dose-dependent influence on chick embryonic development. Significant biochemical alterations were found, including reduction in total protein, albumin, globulin, and most notably alkaline phosphatase levels, which are crucially involved in early differentiation and development of tissues. These results concur with documented toxicological and teratogenic profiles of Venlafaxine in mammalian models and pose questions regarding its effect on embryonic development [18]. The biochemical changes observed indicate that Venlafaxine compromises normal developmental processes, validating its classification as a potential teratogen. Due to its extensive use these findings highlight the need for careful administration throughout pregnancy and call for further studies on its developmental toxicity profile using both avian and mammalian models.

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