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TERATOGENIC AND BIOCHEMICAL EFFECTS OF MODAFINIL ON EMBRYOGENESIS OF GALLUS GALLUS DOMESTICUS

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ABSTRACT

The current study deals with the effects of the nootropic agent Modafinil on embryogenesis of *Gallus gallus domesticus* (White Leghorn strain). Modafinil is a wakefulness promoting used to treat narcolepsy and sleep apnea. No studies on embryotoxicity of Modafinil at molecular and biochemical level has been done in detail as yet. Our research showed that Modafinil induces haemorrhage and severe embryonic malformations at 50mM concentration. Modafinil treated embryos showed changes in enzyme levels (ALP & AChase) as compared to the control embryos. Flow cytometry studies revealed that Modafinil may not induce significant level of apoptosis (Programmed Cell Death) in the developing embryos.

KEYWORDS: Modafinil, Teratogenesis, Chick Embryo, Hamburger-Hamilton (HH) staging system, Protein biochemistry, Flow cytometry.

INTRODUCTION

Hamburger and Hamilton (1951) published photographic series of development of chicken embryo throughout the 21- day incubation period (Hamburger and Hamilton, 1951, 1992; Hamburger, 1992; Sanes, 1992). Romanoff then described the development of the chicken embryonic organ systems throughout the incubation period (Romanoff, 1960) as well as a description of the factors (environmental and some chemical) that can grossly affect embryonic development (Romanoff and Romanoff, 1972).

Modafinil [d, l-2-[(diphenylmethyl) sulfinyl] acetamide), a α1-adrenergic agonist, is a memory-improving and mood-brightening psychostimulant, commonly prescribed in the treatment of narcolepsy and hypersomnia. Modafinil and its R-enantiomer Armodafinil are

central nervous system stimulants used to improve wakefulness in patients with excessive sleepiness. Modafinil increases wakefulness and both have been shown to be helpful in conditions with excessive sleepiness including narcolepsy, obstructive sleep apnea and shift-work sleep disorder.[1] Modafinil was approved for use in the United States in (1998) and Armodafinil in (2007). Their indications are for improvement in wakefulness in adults with excessive sleepiness due to narcolepsy, obstructive sleep apnea, and shift-work disorder. Modafinil increases the risk of congenital embryonic malformations like congenital heart defects, hypospadias, orofacial clefts in human embryos as reported by UK government studies.[2]

Research on Modafinil's wake-promoting mechanism has focused on monoaminergic effects showing Modafinil stimulates histamine (HA), norepinephrine (NE), serotonin (5-HT), dopamine (DA), and orexin systems in the brain.[3] Modafinil's mechanism of action (MOA) is still not fully understood.

MATERIALS AND METHODS

Fertilized and pre-incubated eggs HH stage 0-1[4,5] (0hr+), 12-13 (24h) of *Gallus gallus domesticus* were obtained from Venkateshwara Hatcheries Pvt Ltd, Pune. Freshly prepared sterile solution of 50mM Modafinil (Sun Pharma Laboratories Ltd.) was administered by *in ovo* technique (air sac route) into the developing 4-5 d old chick embryos (n=5). Control and treated embryos were incubated for 24h - 48h at 37.8°C and 70-80% humidity in a BOD incubator.

Post-treatment embryos were harvested in 1X sterile chilled PBS (pH 7.4) and observed for drug- induced anomalies. Observations were recorded under a dissecting binocular Stereo Zoom microscope (Magnus MAZ Series). Permanent slides of treated and control embryos were prepared by the standard micro-technique procedure for detailed observations. Embryo homogenates were prepared using 1X PEB and protein estimation estimation was carried out using Bradford's method (HiMedia, Inc.). Biochemical studies involving enzyme assays (ALP Reagent Kit (Ultichem- Biochemistry kit) and (AChase (Yumizen CA60): Reagent Kit (Delta lab – Butyrlthiocoline method) were carried out on an Auto-analyzer (Yumizen CA60). Animal tissue culture studies using Explant culture of 8-10 day old chick embryo brains was performed using DMEM supplemented with 10% serum and 50mM Modafinil. Explants were observed for cell migration 2-3 days post drug treatment. Conditioned/ spent medium of both control and 50mM treated Modafinil treated explant cultures was removed

and used for protein estimation. Cell viability studies using Trypan Blue Dye Exclusion Assay and Flow cytometry (Dead Cell Apoptosis Kit with FITC Annexin V and PI, for Flow cytometry, Thermo Fisher Scientific, Catalog no. V13242) were carried out for treated and control samples.

RESULTS

Fig. 1: A

Fig. 1: B

Fig.1:A shows control embryo of 50mM Modafinil(24hrs)
Fig.1:B shows control embryo of 100mM Modafinil(48hrs)
Fig.2:A shows treated embryo of 50mM Modafinil(24hrs)
Fig.2:B shows treated embryo of 50mM Modafinil(24hrs)
Fig.2:C shows treated embryo of 100mM Modafinil(48hrs)

Fig. 2: A

Fig. 2: B

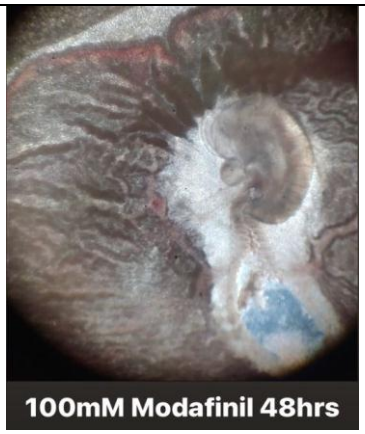


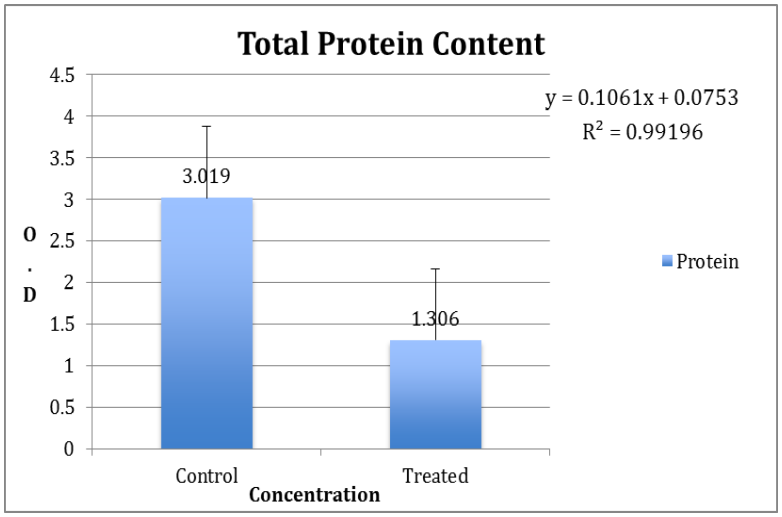
Fig. 2: C

Figure 1: Dose response study for modafinil.

Biochemistry

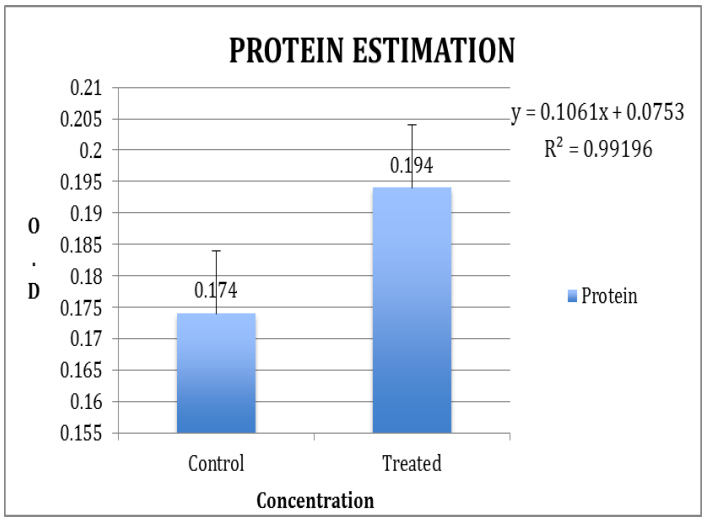
Protein estimation studies for 4 day old 50mM Modafinil.

Sample	Protein (µ/L)
Control	3.019
Treated (50mM)	1.306



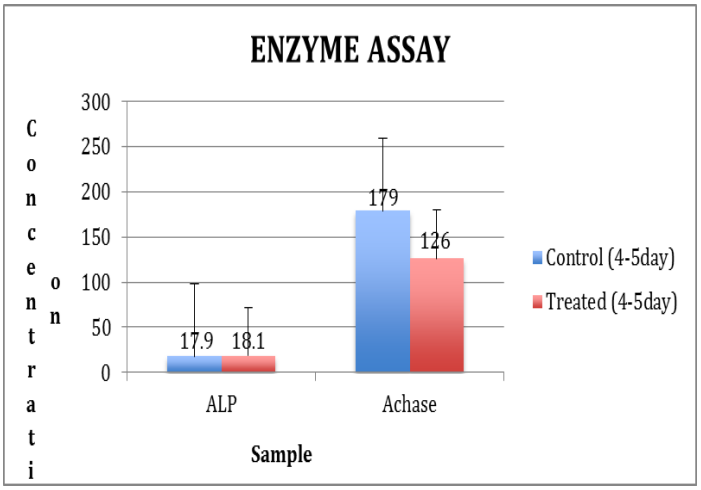
Protein estimation studies for 8 day old 50mM Modafinil

Sample	Protein (µ/L)
Control	0.174
Treated	0.194



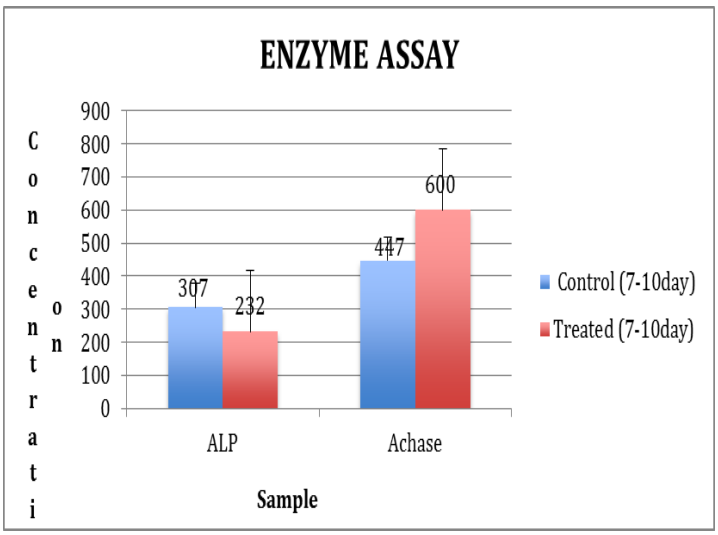
Enzyme assay for 4 day old 50mM Modafinil

Sample	Alp	Achase
1) control (4-5day)	17.9 u/l	179 u/l
2) treated (4-5day)	18.1 u/l	126 u/l



Enzyme assay for 8 day old 50mM Modafinil

Sample	Alp	Achase
1) control (7-10day)	307 u/l	447 u/l
2) treated (7-10day)	232 u/l	600 u/l



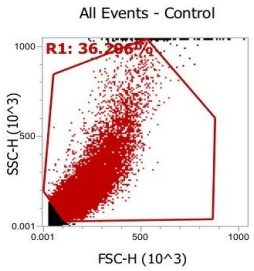
Animal tissue Culture & Cell biology studies

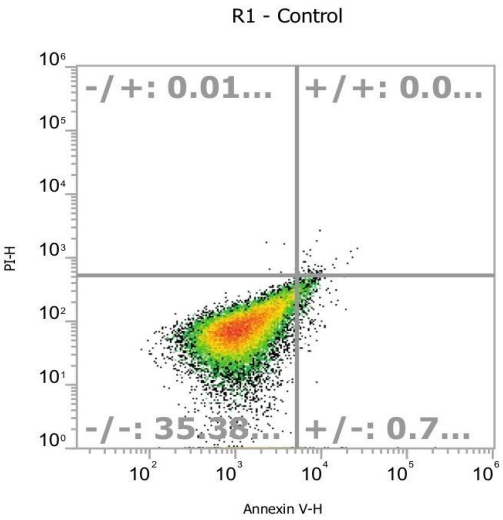
Cell vialbility counting by Trypan Blue Dye Exclusion Assay showed less variation in 50mM Modafinil treated and control samples.

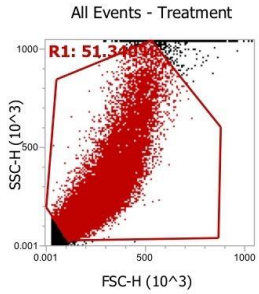
Vailable cell count of treated sample was 96 x 10^4 cells while that of, control sample was 98 x 10^4 cells.

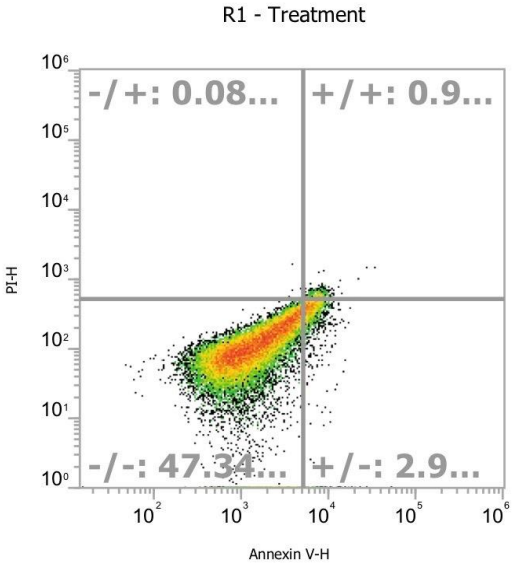
This, indicating that Modafinil may not induce apoptosis at a low dose of 50mM concentration. This result is consitent with the flow cytometry data.

Flow cytometry studies using FITC annexin V and PI staining kit showed almost negligible difference in 50mM Modafinil treated and control embryos.







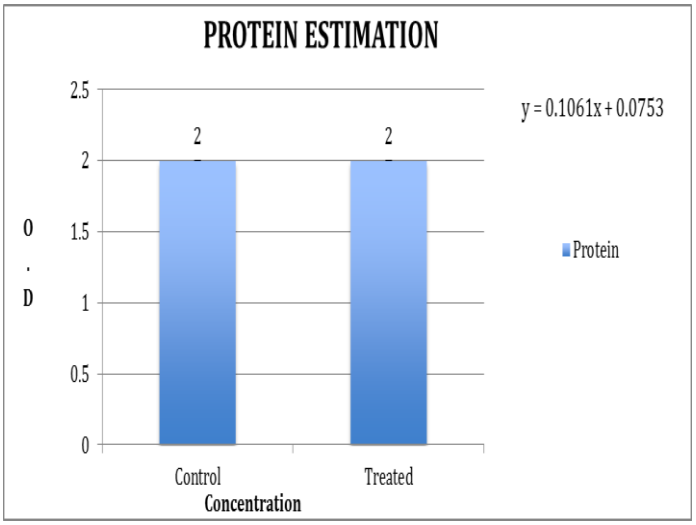


Name	X Parameter	Y Parameter	Count	% Total	% Gated
All Events	N/A	N/A	48,898	100.000	100.000
R1	FSC-H	SSC-H	25,104	51.340	51.340
-/+	BL1-H	BL2-H	43	0.088	0.171
+/+	BL1-H	BL2-H	472	0.965	1.880
-/-	BL1-H	BL2-H	23,151	47.345	92.220
+/-	BL1-H	BL2-H	1,438	2.941	5.728

Name	X Parameter	Y Parameter	Count	% Total	% Gated
All Events	N/A	N/A	69,459	100.00	100.000
R1	FSC-H	SSC-H	25,148	36.206	36.206
-/+	BL1-H	BL2-H	13	0.019	0.052
+/+	BL1-H	BL2-H	68	0.098	0.270
-/-	BL1-H	BL2-H	24,579	35.386	97.737
+/-	BL1-H	BL2-H	488	0.703	1.941

Protein estimation (Bradford Assay) of Spent/ Conditioned medium collected from control and 50mM treated chick embryo brain explant cultures showed identical protein concentration levels.

Sample	Protein (µ/L)
Control	2.0
Treated	2.0



DISCUSSION

Our research showed that 50mM Modafinil treated embryos (7-10 d) showed decrease in ALP (232 U/L) levels compared to control embryos (307 U/L) and significant increseae in AChase levels (600 U/L) as compared to control embryos (447 U/L). However, early stage embryos (4 d) showed decrease in AChase levels (126 U/L: treated V/S 179 U/L: control) and has no significant changes in ALP levels. This results are consistent with earlier studies.[6]

Previous studies have showed that Modafinil stimulated osteogenic differentiation in vitro and increased ALP activity in rats.[7]

Former biochemical studies report alkaline phosphatase levels to be increased post Modafinil treatement of either Modafinil or placebo.[8]

Modafinil (100 mg/kg) was found to significantly potentiate hyperglycaemia and dyslipidaemia but attenuated oxidative stress and AChE activity in diabetic rats.[9]

Our study shows that Modafinil at 50mM concentration does not induce significant level of apoptosis and maybe considered safe for the developing embryo.

However studies performed on hippocampal neurons showed that Modafinil was found to attenuate excessive autophagy and apoptosis both in vitro and in vivo.[10]

These results are synonymous to those where Modafinil treatment provided protection against DA toxicity, cell death, and neuroinflammation [11] . Modafinil (in a dose-dependent way), and the combination of Modafinil and ethanol significantly decreased the brain infarct volume, edema, apoptosis and gliosis in rats with focal cerebral ischemia.[12]

The inhibitory effects of Modafinil on acute pancreatitis and apoptosis is also documented.[13] As mentioned above, our results did not show any such changes.

CONCULSION

The current study showed that drug response is stage dependent and hence, stage specific. Biochemical and differential gene expression studies should be carried out for analysing teratogenecity of nootropic drugs.

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