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Neurotransmitters Level in Hypothyroid Male Albino Rats after Isotretinoin Treatment

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Isotretinoin is the most effective anti-acne treatment but to some extent it has side effects. So, the aim of the present study was focused on the combined effect of isotretinoin treatment and hypothyroidism on cerebral monoamines in male albino rats. The animals were divided into four groups; control, hypothyroid, hypothyroid treated with isotretinoin and isotretinoin only treated. Hypothyroidism was induced by using 5 mg kg^{-1} propylthiouracil for 30 days. Oral isotretinoin was given in olive oil 1.5 mg kg^{-1} b.wt. equivalent to human therapeutic dose for 4 weeks. The withdrawal period is 2 weeks after drug stoppage. The concentration of epinephrine, nor epinephrine, dopamine and serotonin were estimated in different brain areas. Also, the serum level of free triiodothyronine and free thyroxin and thyroid stimulating hormone were measured to insure the hypothyroid state. Total body weight was recorded throughout the experimental period. Hypothyroid rat's model exhibited a decrease of norepinephrine, dopamine and serotonin contents in most of the brain areas examined. Hypothyroid rats treated with isotretinoin exhibited elevated levels of neurotransmitter in most brain areas. isotretinoin treatment does not aggravate the monoamines dysfunction in hypothyroid rats.

Key words: Isotretinoin, hypothyroidism, neurotransmitters, acne

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INTRODUCTION

Acne vulgaris is the commonest skin disease that affect 80-90% of adolescent aged 12-14 years (Stern, 1992). Adolescent stage is a complex life cycle characterized by many striking biological, psychological, physical and social changes (Dalgard *et al*., 2008; Huang, 2010). In 1982, an effective treatment of acne was introduced by the oral route known as isotretinoin, an attempt to improve biological activity and minimize side effects of vitamin A compounds that were used as an effective treatment prior to the development of isotretinoin (Kontaxakis *et al*., 2009).

Isotretinoin which naturally occur in the body is retinoid derived from vitamin A, that are essential in regulating the function of multiple organ systems in embryonic and adult mammals (Maden, 2000). Isotretinoin is the cis-configuration of tretinoin, which is acid form of vitamin A. Vitamin A and its analogue all-trans retinoid bind to specific nuclear receptor (retinoid acid receptors) and alter gene expression leading to their biological effects (Mangelsdorf *et al*., 1993).

The recommended oral dose from isotretinoin for acne treatment is 0.5-1.0 mg kg^{-1} day^{-1}. During treatment, the dose may be adjusted according to the response of the disease and on the appearance of clinical side effects (Jones *et al*., 1980; Pochi *et al*., 1991). Isotretinoin treatment should continue for 15-24 weeks or until the total cyst, the count has decreased by over 70% (Jones *et al*., 1980; Sweetman, 2011). It is widely used also in acne patients suffering from various diseases such as hypothyroidism. The adverse events related to depression suicidal idea and psychosis have been more common with isotretinoin than with other acne treatment such as antibiotics (Mano *et al*., 1998; Wysowski *et al*., 2001).

Studies in mice and rats indicated that retinoid cross the blood brain barrier into central nervous system (Le Dose *et al*., 2000) and their receptors were found in adults brain, they may alter neural pathway such as dopamine signaling, which was known to be involved in mood and thought disorders (Krezel *et al*., 1998).

Reports of Mey and Mccaffery (2004) stated that retinoid receptors are present in brain and in turn retinoid acid plays a crucial role in brain development and the continued expression of retinoid acid receptors in the adult brain suggested ongoing function of this signaling pathway. In adults, retinoid acid receptors are known to control a large numbers of genes and retinoic acid can signal neural cells to become neurons (Lane and Bailey, 2005). The thyroid hormones are essential for cellular metabolism, growth and differentiation of several organs including the brain (Bernal, 2002; Yen, 2001). Thyroid hormones and retinoid are not only essentials for proper development of the central nervous system (Maden, 2002; De Escobar *et al*., 2004) but also for adult brain maintenance (Bianco *et al*., 2002; Lane and Bailey, 2005). Even thyroid hypothyroxinemia during pregnancy has been shown to impair proper neural migration in the somatosensory cortex and hippocampus in rodents (Lavado-Autric *et al*., 2003). Cattani *et al*. (2013) stated that congenital hypothyroidism is associated with the delay in cell migration and proliferation in brain tissue, impairment of synapse formation, misregulation of neurotransmitters, hypomyelination and mental retardation.

Thyroid Hormones (THs) regulate the neural cytoarchitecture, the normal growth and the synaptogenesis and their receptors are widely distributed in the central nervous system (Esposito *et al*., 1997; Henley and Koehnle, 1997). Thyroid Hormone Receptors (THRs) are members of a large superfamily of nuclear receptors including those of estrogens, glucocorticoids and retinoic acid (Weinberger *et al*., 1986).

Adult onset thyroid dysfunction is associated with both neurological and behavioral abnormalities (DeGroot *et al*., 1984) emphasizing the importance of THs for normal brain function.

Therefore, the aim of this study was to evaluate the influence of oral isotretinoin (Iso) treatment on the content of norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5HT) in different brain areas (cortex, striatum, hippocampus, midbrain and cerebellum) of adult male hypothyroid rat model after 2 and 4 weeks and after Withdrawal Period (WP) 2 weeks after drug stoppage. The study also aims to elucidate the relationship between the thyroid function and cerebral monoamines.

MATERIALS AND METHODS

Study groups: One hundred and twenty-eight Wister male albino rats weighing 200-250 g were used for this study. The animals were housed in a temperature (25±1°C), humidity controlled room and a 12 h light-dark cycle (lights on at 06:00 h). Rats were allowed free access to water and standard pellet diet. The institutional animal ethics committee approved all experimental protocols. The animals were classified into four groups, each of 32 rats as follows:

Control group (C): Rats received oral daily dose of olive oil for four weeks.

Hypothyroid group (H): The hypothyroid rats status was proved by measurement of Free Triiodothyronine (fT3) and Free Thyroxin (fT4) as well as Thyroid Stimulating Hormone (TSH) in serum by ELIZA kits according to the method of Wenzel (1981), Braverman (1996) and Midgley (2001), respectively. Induction of hypothyroidism by Propylthiouracil (PIU) 5 mg kg^{-1} b.wt. oral daily dose for 30 days (Serakides *et al*., 2002; Silva *et al*., 2004). The development of hypothyroid is considered zero time.

Hypothyroid treated with isotretinoin (H+Iso): Animals received oral daily dose of isotretinoin 1.5 mg kg^{-1} b.wt. for 4 weeks. Hypothyroid state was developed as group (H). The development of hypothyroid is considered zero time.

Healthy animals treated with isotretinoin (Iso): Animals received oral daily dose of isotretinoin 1.5 mg kg^{-1} b.wt. for four weeks.

Drug used: The isotretinoin drug (Iso) is Roaccutane (Roche) tablets. It was obtained from F. Hoffmann-LaRoche-Ltd-Switzerland by R.P.Scherer Gmbh Co. KG; Eberbach; Germany. Each capsule contains 20 mg isotretinoin diluted in olive oil. The rat dose was 1.5 mg kg^{-1} day^{-1}, which is equivalent to the human therapeutic dose converted to rat dose according to (Paget and Barners, 1964).

The dose was given daily for four weeks to both H+Iso and Iso groups by stomach tube 2 h before the supply of food to insure proper adsorption of the drug (after the development of hypothyroid which is considered the zero time). Drug dosing was stopped by the end of four weeks and animals left untreated for extra two weeks for the withdrawal of the drug (W.P).

Observation: Food intake and locomotor activity were observed daily for 4 weeks following isotretinoin treatment and after two week of drug cessation. The observations declared that isotretinoin treated rats exhibited a decrease in food intake and locomotor activity when compared with control. However, after withdrawal period (two weeks), food intake and locomotor activity slightly improved.

Body weight monitoring: Animals in all groups were weighed at zero time, 2 weeks, 4 weeks as well as 2 weeks after stoppage of Isotretinoin treatment (W.P).

Blood sampling: Blood samples were withdrawn from retro-orbital plexus (Schemer, 1967) at zero time, 2, 4 and 2 weeks after drug stoppage (W.P). Serum was separated for the determination of FT3 and FT4 as well as TSH to insure the continuous presence of hypothyroid status as shown in the Table 1 and Fig. 1.

Handling of tissue samples: Eight animals were decapitated at 0, 2, 4 and 2 weeks after stoppage of drug treatment (W.P). Decapitation was performed at 10.00 am. Brains were rapidly excised and transferred to ice cold glass plate and dissected into the following areas; cortex, striatum, hippocampus, midbrain and cerebellum. All tissues were plotted dry then used for the determination of neurotransmitters.

Estimation of neurotransmitters: Epinephrine, nor epinephrine, dopamine and serotonin were estimated by a validated HPLC method (Peat and Gibb, 1983).

Statistical analysis: Results were shown as Mean±SE for each group. Statistical analysis was performed using SPSS 9.0. For multiple comparisons, one-way

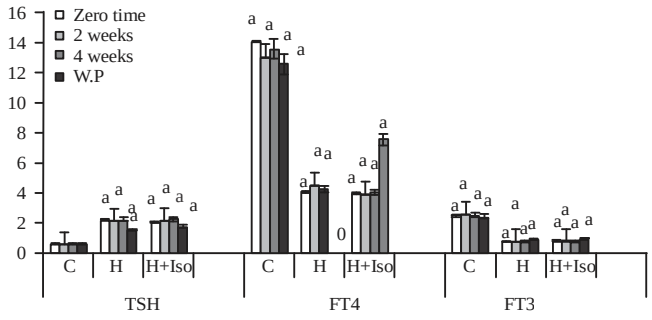

Fig. 1: Level of TSH, fT4 and fT3 (mIU mL^{-1}, Pg mL^{-1} and Pg mL^{-1}) in serum

Table 1: Effect of Isotretinoin (1.5 mg kg^{-1}+b.wt. daily oral administration, for 4 weeks) on TSH, fT4 as well as fT3 (mIU mL^{-1}, Pg mL^{-1} and Pg mL^{-1}) in serum

Groups	Zero time	2 Weeks	4 Weeks	W.P
TSH				
C	0.55±0.05	0.58±0.04	0.55±0.03	0.56±0.05
H	2.2±0.25[a]	2.1±0.20[a]	2.2±0.24[a]	1.5±0.13[a]
	(+300%)	(+262.06%)	(+318.18%)	(+167.85%)
H+ Iso	2.0±0.18 [a]	2.1±0.2[a]	2.2±0.19[a]	1.7±0.12[a]
	(+263.63%, -9.09%)	(+262.06%, 0.0%)	(+300.00%, -4.3%)	(+203.35%, -13.3%)
FT4				
C	14.00±0.88	13.00±1.4	13.5±1.0	12.5±1.4
H	4.0±0.9[a]	4.5±0.7[a]	4.2±0.8[a]	±1.2[a]
	(-71.4%)	(-65.3%)	(-68.8%)	(-36.0%)
H+ Iso	3.9±0.25[a]	3.9±0.4[a]	4.0±0.2[a]	7.5±0.8[a]
	(-72.4%, -2.5%)	(-70%, -13.33%)	(-70.3%, -4.76%)	(-40.0%, -6.25%)
FT3				
C	2.4±0.15	2.6±0.9	2.5±0.8	2.4±0.9
H	0.72±0.01[a]	0.75±0.03[a]	0.77±0.03[a]	0.9±0.09[a]
	(-70%)	(-71.15%)	(-69.2%)	(-64%)
H+Iso	0.8±0.09[a]	0.77±0.0.01[a]	0.72±0.06[a]	0.9±0.08[a]
	(-66.66%, +11.11%)	(-70.38%, +2.66%)	(-71.2%, -6.49%)	(-64.0%, 0%)

Values are Mean±SEM and percent of change, [a,b]Mean differ significantly ($p<0.05$) from control and hypothyroidism model, C: Control, Hypothyroidism, H+Iso: H: Hypothyroidism+isotretinoin, Iso: Isotretinoin

analysis of variance (ANOVA) was used. In cases where ANOVA showed significant difference, *post hoc* analysis was performed with least significant. The $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of isotretinoin on norepinephrine (NE) and epinephrine (E): As illustrated in Table 2 and comparing to control group, the NE level in different brain areas of group (H) rats (cortex, striatum, mid brain, hippocampus and cerebellum); there is significant decrease in the NE after 2 and 4 weeks. After the treatment with isotretinoin; the level of NE in H+Iso group start to increase in most of the areas. As compared to control, Iso treated group there is an increase in NE after 4 weeks of treatment. After withdrawal period the NE level still exist above the normal level in brain tissues of all groups.

Table 3 illustrated the effect of isotretinoin on E level; from data we observed that there is no significant change in E level in hypothyroid group throughout the experimental period. But with isotretinoin treatment the level of E increased in cortex and striatum and hippocampus while in mid brain there is no change but in cerebellum there is a decrease compared by H group. With the withdrawal of the drug, the epinephrine level start to return to the normal level but also increased to some extant in hippocampus and cortex so, the study revealed that the withdrawal period should be extended for more than two weeks.

Effect of isotretinoin on DA and 5 HT: As shown in Table 4 compared to control; the dopamine level in cortex and striatum and hippocampus was increased significantly in group H and group Iso after 2 and 4 weeks as well as after treatment stoppage.

Regarding the midbrain and cerebellum, DA level was decreased in group H but increased in group Iso as compared to control. The 5HT level was significantly decreased in all brain areas of hypothyroid group H; throughout the experimental time.

But after the treatment, this group with isotretinoin H+Iso the level was elevated significantly in all brain tissues except hippocampus and striatum which still exhibited significant decrease. During withdrawal period the level of serotonin begin to increased and exceed the normal level (Table 5).

The DA level in group Iso was significantly decreased in cortex and striatum as well as hippocampus comparing to control group but there was no change in mid brain and cerebellum significantly.

Effect of isotretinoin on body weight: As shown in the Table 6 and Fig. 2, the hypothyroid model rats exhibited significantly decrease in total body weight as compared with control rats. Meanwhile, H+Iso group exhibited decrease body weight compared to control group but didn't show any significant change compared to the hypothyroid model. The body weight of animals group Iso decrease after 2 and 4 weeks of administration. But after W.P, the body weight didn't reach the control value which indicated that 2 weeks is not enough for withdrawal of the drug.

Table 2: Effect of Isotretinoin (1.5 mg kg^{-1} b.wt. daily oral administration, for 4 weeks) on norepinephrine levels (µg g^{-1} tissue) in different brain area

	Norepinephrine (µg g^{-1} tissue)			
Brain area and groups	Zero time	2 Weeks	4 Weeks	W.P
Cortex				
C	0.141±0.004	0.144±0.002	0.175±0.009	0.174±0.029
H	0.115±0.004	0.114±0.001	0.144±0.008	0.173±0.012
H+ Iso		0.119±0.004	0.169±0.012^{b}	0.191±0.014^{ab}
Iso		0.128±0.002^{ab}	0.200±0.010^{ab}	0.181±0.008^{ab}
Striatum				
C	0.140±0.002	0.133±0.005	0.129±0.007	0.125±0.018
H	0.069±0.003^{a}	0.068±0.005^{a}	0.103±0.005^{a}	0.099±0.015^{a}
H+ Iso		0.067±0.005^{ab}	0.111±0.006^{a}	0.119±0.003^{ab}
Iso		0.067±0.004^{ab}	0.148±0.008^{ab}	0.109±0.009^{a}
Hippocampus				
C	0.160±0.001	0.162±0.001	0.088±0.004	0.089±0.007
H	0.064±0.001	0.062±0.002	0.091±0.006	0.101±0.019
H+ Iso		0.065±0.001	0.081±0.004^{ab}	0.088±0.014^{b}
Iso		0.061±0.002	0.125±0.009^{ab}	0.095±0.016^{b}
Mid brain				
C	0.058±0.003	0.055±0.001	0.056±0.003	0.056±0.005
H	0.038±0.002^{a}	0.041±0.003^{a}	0.047±0.003	0.057±0.013
H+ Iso		0.040±0.002^{a}	0.044±0.003^{a}	0.063±0.006
Iso		0.042±0.002^{a}	0.106±0.007^{ab}	0.062±0.008
Cerebellum				
C	0.098±0.004	0.085±0.001	0.082±0.005	0.087±0.011
H	0.063±0.005^{a}	0.062±0.006^{a}	0.076±0.004	0.101±0.003
H+ Iso		0.062±0.004^{ab}	0.085±0.004	0.074±0.019^{b}
Iso		0.060±0.006^{ab}	0.118±0.006^{ab}	0.087±0.011^{b}

Values are Mean±SEM , ^{a,b}Mean having different superscript letters in the same row within the same brain area differ significantly ($p<0.05$) from control and hypothyroid model, C: Control, H: Hypothyroidism H+Iso: Hypothyroidism+is Isotretinoin, Iso: Isotretinoin

Table 3: Effect of isotretinoin (1.5 mg kg^{-1} b.wt. daily oral administration, for 4 weeks) on epinephrine levels in different brain area

	Epinephrine (µg g^{-1} tissue)			
Brain area and groups	Zero time	2 Weeks	4 Weeks	W.P
Cortex				
C	0.225±0.014	0.197±0.010	0.226±0.012	0.221±0.021
H	0.226±0.014	0.207±0.014	0.197±0.012	0.213±0.005
H+Iso		0.211±0.013^{a}	0.215±0.017^{b}	0.243±0.007^{ab}
Iso		0.201±0.01b	0.221±0.01b	0.228±0.006
Striatum				
C	0.062±0.003	0.057±0.004	0.058±0.004	0.073±0.005
H	0.059±0.003	0.051±0.003	0.057±0.004	0.061±0.004
H+Iso		0.055±0.004	0.066±0.004^{ab}	0.056±0.006^{a}
Iso		0.059±0.005^{b}	0.065±0.007^{ab}	0.059±0.004^{a}
Hippocampus				
C	0.169±0.010	0.171±0.008	0.162±0.014	0.157±0.019
H	0.159±0.006^{a}	0.168±0.009	0.106±0.023	0.107±0.027
H+Iso		0.153±0.008^{ab}	0.154±0.015^{a}	0.168±0.017^{b}
Iso		0.168±0.009	0.127±0.009^{ab}	0.138±0.017^{ab}
Mid brain				
C	0.028±0.003	0.027±0.002	0.023±0.001	0.029±0.002
H	0.025±0.002	0.032±0.001	0.021±0.003	0.028±0.003
H+Iso		0.030±0.002	0.022±0.002	0.027±0.001
Iso		0.030±0.002	0.019±0.004	0.028±0.002
Cerebellum				
C	0.123±0.007	0.109±0.005	0.105±0.021	0.096±0.011
H	0.115±0.004	0.113±0.004	0.125±0.026	0.128±0.019
H+Iso		0.116±0.01	0.085±0.01^{b}	0.115±0.025^{a}
Iso		0.060±0.006	0.118±0.011^{ab}	0.087±0.011^{b}

Values are Mean±SEM, ^{a,b}Mean having different superscript letters in the same row within the same brain area differ significantly ($p<0.05$) from control and hypothyroid model, C: Control, H: Hypothyroidism H+Iso: Hypothyroidism+is Isotretinoin, Iso: Isotretinoin

Table 4: Effect of Isotretinoin (1.5 mg kg^{-1} b.wt. daily oral administration, for 4 weeks) on dopamine levels (µg g^{-1} tissue) in different brain area

	DA µg g^{-1} tissue			
Brain area and grous	Zero time	2 Weeks	4 Weeks	W.P
Cortex				
C	0.547±0.041	0.559±0.029	0.691±0.069	0.600±0.013
H	0.400±0.033[a]	0.402±0.028	0.868±0.054	0.862±0.064
H+Iso		0.533±0.034[a]	0.817±0.026[ab]	0.709±0.083[ab]
Iso		0.617±0.028[ab]	0.865±0.121[a]	0.786±0.025[ab]
Striatum				
C	0.423±0.027	0.371±0.011	0.352±0.043	0.350±0.042
H	0.300±0.019	0.399±0.018	0.400±0.061[a]	0.536±0.035[a]
H+Iso		0.436±0.027[ab]	0.404±0.064[a]	0.351±0.052[b]
Iso		0.412±0.026[ab]	0.452±0.043[a]	0.444±0.043[a]
Hippocampus				
C	0.235±0.005	0.237±0.007	0.493±0.064	0.490±0.098
H	0.180±0.008[a]	0.248±0.011[a]	0.622±0.065	0.602±0.072
H+Iso		0.255±0.003[ab]	0.562±0.095[ab]	0.459±0.061[ab]
Iso		0.250±0.009[ab]	0.562±0.085[ab]	0.532±0.065[b]
Mid brain				
C	0.145±0.011	0.143±0.013	0.145±0.014	0.150±0.015
H	0.130±0.005[a]	0.138±0.006	0.135±0.018	0.165±0.021
H+Iso		0.146±0.005	0.159±0.024[a]	0.151±0.012[ab]
Iso		0.159±0.012[ab]	0.162±0.005[b]	0.158±0.009[a]
Cerebellum				
C	0.246±0.009	0.307±0.021	0.368±0.046	0.360±0.009
H	0.207±0.013[a]	0.280±0.024[a]	0.285±0.047[a]	0.280±0.038[a]
H+Iso		0.277±0.017[a]	0.376±0.031[b]	0.381±0.056[a]
Iso		0.379±0.013[ab]	0.400±0.039[ab]	0.384±0.034[a]

Values are Mean±SEM , [a,b]Mean having different superscript letters in the same row within the same brain area differ significantly ($p<0.05$) from control and hypothyroid model, C: Control, H: Hypothyroidism H+Iso: Hypothyroidism+is Isotretinoin, Iso: Isotretinoin, DA: Dopamine

Table 5: Effect of Isotretinoin (1.5 mg kg^{-1} b.wt. daily oral administration, for 4 weeks) on levels (µg g^{-1} tissue) in different brain area

	5HT µg g^{-1} (tissue)			
Brain area and groups	Zero time	2 Weeks	4 Weeks	W.P
Cortex				
C	0.188±0.011	0.174±0.009	0.195±0.027	0.192±0.024
H	0.174±0.013[a]	0.125±0.008[a]	0.101±0.035[a]	0.132±0.032[a]
H+Iso		0.171±0.01[b]	0.165±0.027[ab]	0.216±0.029[ab]
Iso		0.169±0.012[b]	0.139±0.012[ab]	0.174±0.018[abc]
Striatum				
C	0.077±0.003	0.083±0.005	0.086±0.005	0.056±0.009
H	0.056±0.004[a]	0.066±0.007[a]	0.062±0.003[a]	0.056±0.012
H+Iso		0.041±0.005[b]	0.049±0.008[ab]	0.089±0.004[ab]
Iso		0.042±0.004[b]	0.043±0.005[ab]	0.084±0.006[ab]
Hippocampus				
C	0.083±0.004	0.053±0.008	0.083±0.009	0.071±0.007
H	0.060±0.006[a]	0.068±0.009[a]	0.069±0.015[a]	0.077±0.018
H+Iso		0.048±0.008[b]	0.039±0.009[ab]	0.079±0.010[a]
Iso		0.058±0.009[b]	0.056±0.015[ab]	0.075±0.005[a]
Mid brain				
C	0.049±0.003	0.044±0.006	0.052±0.009	0.038±0.001
H	0.050±0.004	0.039±0.003	0.037±0.006[a]	0.041±0.006
H+Iso		0.052±0.005	0.035±0.004[a]	0.058±0.011[ab]
Iso		0.046±0.004	0.054±0.006[b]	0.049±0.005[ab]
Cerebellum				
C	0.061±0.007	0.064±0.004	0.066±0.013	0.075±0.002
H	0.029±0.002	0.059±0.005	0.052±0.008	0.064±0.007
H+Iso		0.067±0.003	0.061±0.009	0.071±0.015
Iso		0.061±0.007	0.059±0.010	0.067±0.011

Values are Mean±SEM, [a,b]Mean having different superscript letters in the same row within the same brain area differ significantly ($p<0.05$) from control and hypothyroid model, C: Control, H: Hypothyroidism H+Iso: Hypothyroidism+is Isotretinoin and Iso: Isotretinoin, 5HT: Serotonin

Table 6: Body weight changes of control and test group's throughout the experimental period

Body weight (g)	Zero time	2 Weeks	4 Weeks	W.P
C	230.5±7.5	235.4±7	252.3±10.5	262.5±11
H	160±8.5[a]	162.5±9.5[a]	165±12[a]	175±14.5[a]
	(-30.4%)	(-34.15%)	(-40.3%)	(-33.33%)
H+ Iso	160±11[a]	155±10[a]	150.6±13.3[a]	175±16.5[a]
	(-30.4%, 0%[a])	(-34.15%, -0.05%)	(-40.3%, -0.09%)	(-33.33%, 0%)
Iso	230.0±8.5[b]	209±11[ab]	190±13.5[a]	212.5±12[ab]
	(0%, +43.5%)	(-11.21%, +28.6%)	(-24.96%, +0.15%)	(-19.04%, +2.14%)

Data presented Mean±SEM and percent of change, [a,b]Mean differ significantly ($p<0.05$) from control and hypothyroid model, C: Control, H: Hypothyroidism, H+Iso: Hypothyroidism+ isotretinoin, Iso: Isotretinoin

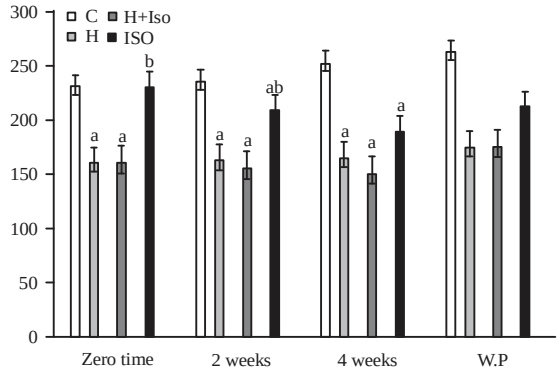

Fig. 2: Body weight changes during the experimental period

DISCUSSION

The evidence from the literature hence show that isotretinoin treatment affect individual in different ways, but even though the diversity and severity of symptoms vary, all have adverse effects on mental well-being of these individuals. The physiological effects of isotretinoin are presumably on pathways that are part of pathology of these psychiatric conditions, but with the most prominent effects being on those pathways that engender depression (Yen, 2001).

Several studies in animal models have shown that 13-cis-retinoic acid induced an increase in depression-related behavior (O'Reilly *et al*., 2006, 2008). The hypothyroid rat model (Serakides *et al*., 2002; Silva *et al*., 2004) in this study presented by significant decrease in serum fT4 and fT3 and highly significant increase of TSH level throughout the experimental period (Table 1).

There is a significant decrease in body weight of hypothyroid rat model used Table 6 compared to control group which coincide with the results of Schneider and Golden (1987), Dratman *et al*. (1987) and Silva *et al*. (2004). In thyroid hypofunction, an increase of protein catabolism occurred, with consequent reduction of muscle mass; synthesis of proteins, vitamins, growth factors; reduction of intestinal absorption of carbohydrates; in addition to osteopenia (Allain *et al*., 1995).

Change *et al*. (2014) demonstrated that chemical destruction of the thyroid gland by PTU alters hematopoiesis, the secretion of gastric inhibitory peptide and steroidogenesis. In regard to growth-related molecules, the active ghrelin and total ghrelin secretion was enhanced and the expression of GHS-R was up-regulated in hypothyroid rats. However, IGF-1 secretion in hepatocytes was inhibited by hypothyroidism. The IGF-1 has growth-promoting effects on almost all cells in the body, particularly skeletal muscle, bone, cartilage, liver, kidney, nerve, skin, hematopoietic tissue and lung cells.

However, Mano *et al*. (1998) reported that there was no difference in body weight between hypothyroid and control rats. Changes in body mass of animals with thyroid dysfunction are probably associated with a longer period of disease occurrence and the induction period has not allowed the observation of such occurrence in Mano *et al*. (1998) study. As regards the W.P. (two weeks after stoppage of Isotretinoin treatment) the body weight of group H and H+Iso tended to regain body weight compared to C group. In the same period (W.P.), fT4 showed an increased level probably due to regeneration of some thyroid acini, but there was no increase in fT3 which may be due to inactivation of de-iodinase enzyme (Bahls and De Carvalho, 2004). In hypothyroid rats there are was a marked decrease of NE and DA as well as 5HT contents in most of the brain area examined (Table 2-5). Brain serotonin synthesis and turnover in rats was decreased in the sensitivity of serotonin receptors (Vaccari, 1982) and compensatory increase in the density of 5HT receptors, secondary to reduction in the level of synaptic serotonin (Tejani-Butt *et al*., 1993). Immunohistochemical studies in animal brains showed that T3 has high concentration in sinaptosomes, especially those located in noradrenergic neurotransmission brain nuclei (Dratman and Gordon, 1996).

Henley and Koechnle (1997) mentioned that in rat brains there is a slight decrease in cortical density of beta, alpha 1 and alpha 2 receptors in hypothyroidism. The decrease in the thyroid activity of adrenergic post synaptic

receptors, causing a functional decrease in nor adrenergic neurotransmission (Hendrick *et al*., 1998).

Results of the present study showed that daily oral administration of Isotretinoin (1.5 mg kg^{-1} day^{-1}) for 4 weeks produced significant changes in the neurotransmitters (NE and E, DA and 5 HT) content in some of the brain areas examined (cortex, Striatum, hippocampus, midbrain and cerebellum) in both healthy Iso and H+Iso groups.

As regards H+Iso group, results denoted that changes in contents of E and NE in most of brain area examined, it is more pronounced in the 4 weeks of treatment (Table 2 and 5). There is an increase in the content of DA accompanied by a decrease in serotonin content in most of the brain area examined (Table 3 and 4). The changes in the contents of neurotransmitters NE and DA as well as 5 HT still existed in the W.P. of both H+Iso and Iso groups but it does not return to control level which indicate that the W.P. should extended more than 2 weeks. Several reports have described isotretinoin effect on the rodent central nervous system (Ferguson *et al*., 2005; O'Reilly *et al*., 2006). Identification of retinoid receptors in various brain regions of adult animals indicated a functional role for retinoic acid in adult (Krezel *et al*., 1998; Le Dose *et al*., 2000).

The present results also indicated that the alteration in brain regions contents of neurotransmitters by daily oral administration of isotretinoin for 4 weeks was accompanied by symptoms, such as decreased body weight, food intake and locomotor activity, finding that maybe interpreted as signs of depression in rodents (D'Aquila *et al*., 2000). These effects were not resolved completely by the end of W.P. Other investigators (Booij *et al*., 2003; Ruhe *et al*., 2007) mentioned that dysfunction in monoamines systems of DA, NE and 5 HT may be related to depressive disorders. Retinoid are known to bind to their receptors in the brain and to exert effects on gene transcription. Retinoid receptors are concentrated in the limbic area that have been associated with depression, including the amygdala, prefrontal cortex and hippocampus. Retinoid also influence neurochemical system that have been implicated in depression, in particular, dopamine but to some extent serotonin and nor epinephrine (Bremner and McCaffery, 2008). According to Bremner and McCaffery (2008), these effects can be translated into behavioral effects, including symptoms of affective disorders.

Decreased neurogenesis has been also associated with mood disorders, including depression (Bremner *et al*., 2012; Sheline *et al*., 1996). In adult neurogenesis, where new neurons proliferate and become functionally integrated with existing neurons has been most widely studied in the hippocampus (Kempermann *et al*., 2004a, b).

Ferguson *et al*. (2005) Examined the effect of 13-Cis RA treatment on monoaminergic system in adult rats; in this study found no effect on 5 HT or 5HIAA content in brain tissue homogenates of either hippocampus or frontal cortex, 5HIAA levels in the striatum were increased in male rats administered 13-Cis RA. These indicate recycling or reuptake of 5HT in this study.

Hypothalamus is the hormone regulatory center of the brain and a part of Hypothalamus/Pituitary/Adrenal (HPA) axis, it is a central component in the response to stress, also hypothalamus is again apart hypothalamus/pituitary/thyroid axis which regulate the release of TSH and THs.

One particular RA regulated gene in the hypothalamus that may provide a link between RA and depression is Corticotrophin-Releasing Hormone (CRH) (Arborelius *et al*., 1999). This result further emphasizes the importance of Retinoid Acid (RA) in the hypothalamus and the potential for overlap between RA regulated hypothalamic pathways and those that underline depression.

In psychiatric population, the rate of clinical hypothyroidism ranges from 0.5-8% (Targum *et al*., 1984) although in cases of refractory depression the rate of this thyroid dysfunction reaches more than 50% (Howland, 1993). Also, the result of Pies (1997) suggested that a thyroid dysfunction may represent a pathophysiological phenomenon in subgroups of depressed patients.

A high affinity of T3 binding sites in rat brain spatial and regional distribution pattern of thyroid hormone receptors and mRNA have been described in brain at both development and adult stages (Bradley *et al*., 1989; Cook *et al*., 1992; Mellstrom *et al*., 1991).

Also the DNA Binding Doman (DBD) enable TRH to bind to sites known as Thyroid Responsive Element (TRE) located within the promoter region of target gene, THRs may bind with a necessary protein such as retinoid acid (Glass, 1994). Retinoids are well-known regulators of neurons system development, outgrowth and connectivity (Maden, 2002). Adolescence and puberty are times of significant developmental changes in the brain, particularly in relation to motivational and emotional behaviors, that could make this age group particularly vulnerable to the effects of retinoids (Spear, 2000).

Numerous behavioral studies in animal models have pointed to behavioral changes in rodents when the balance of RA is disrupted, either from exposure to excess or deficiency (Bremner *et al*., 2012; Olson and Mello, 2010). Finding in our study suggested that isotretinoin treatment in both healthy (Iso group) and hypothyroid animals H+Iso may affect brain monoamines system, providing a possible biological mechanism by which isotretinoin treatment could lead to depressive disorders.

Further studies investigating the metabolic effect of isotretinoin and depression also using quantitative signs of depression in validated behavioral assessment to confirm stages of depression in isotretinoin treated animals.

Depression is one of the most common psychiatric illnesses and it may interfere significantly with a patient's daily functioning and quality of life. Depression occurring secondary to medications is similar in presentation to endogenous depression and carries similar risks of morbidity and mortality. While the overall prevalence is unknown, drug-induced depression poses a significant challenge for practitioners, as it may undermine the effectiveness of much-needed treatment.

However, some interventions may prove useful, Identifying patients with risk factors (e.g., history of a depressive episode) and implementing a prospective monitoring plan seems prudent, but will not prevent drug-induced depression. Careful monitoring and early detection may, however, minimize the negative sequelae associated with depression.

So, our study recommended that; because of its potency and its possible side effects, isotretinoin is reserved only for those with severe inflammatory or cystic acne that has failed to respond to other treatment options. All patients taking isotretinoin are kept under careful monitoring by their doctors as well as treating psychosocial stressors with interventions such as Pretreatment with selective serotonin reuptake inhibitor may also prove beneficial.

CONCLUSION

The study concludes that the hypothyroidism exhibited lowering the level of neurotransmitters in most of the brain tissues. However, the treatment with isotretinoin, which is the most effective anti-acne treatment not aggravates the status but lead to an elevation of E and NE and DA and 5 HT level in brain tissues under study.

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