

# Propylthiouracil Induced Neurotoxicity in Swiss Albino Mice Fetus

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## ABSTRACT

**Introduction:** Anti-thyroid drugs are used to treat hyperthyroidism in pregnancy, as pregnancy-associated hyperthyroidism causes fetal and maternal complications. Propylthiouracil is recommended for the treatment of hyperthyroidism in the first trimester, though limited reports of congenital anomalies are associated with its antenatal usage. The present study aims to reveal the teratogenicity of propylthiouracil by studying its neurotoxic effect in mice fetuses.

**Material and Methods:** Propylthiouracil was orally administered in a dose of 100 mg/kg body weight/day to pregnant Swiss albino mice during the period of organogenesis (treated group), while pregnant dams of control group were given distilled water orally (same volume and for same duration). The pregnancy was terminated on the 18th day of conception after sacrificing the pregnant dams and fetuses were collected. The fetal brains (from both, control and treated groups) were dissected out, examined and processed for further histological study.

**Results:** The brains of the treated group shows hemorrhagic spots on gross examination, while microscopic examination shows degeneration in the hippocampal area, degenerated choroid plexus and dilatation of lateral and fourth ventricles along with the destruction of ependymal lining of these ventricles. No such findings are present in brain of control group.

**Conclusion:** The safety of propylthiouracil must be re-evaluated through large randomised clinical trials, as it is observed to have neurotoxic effect on mice fetus in the present study

**Keywords:** Choroid Plexus, Hyperthyroidism, Neurotoxic effect, propylthiouracil.

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## INTRODUCTION

The science that deals with the study of congenital malformations caused due to anomalous growth and differentiation in fetus when a pregnant female is exposed to certain exogenous agents are called teratology. Documentation of these teratogenic agents is critical, to ensure a decrease in risk and incidence of congenital abnormalities, as they are generating huge liability for any country in terms of expenditure and also as a poor health indicator. Cognizance about these agents would enable medical experts to restrict their fetal exposure and hence congenital abnormalities.<sup>1</sup>

Hyperthyroidism during pregnancy causes increased maternal and fetal complications like abortion, pre-eclampsia, thyroid storm, premature birth, low birth weight and fetal death.<sup>2,3</sup> The commonest cause of pregnancy-associated hyperthyroidism is Graves' disease.<sup>4</sup> In Graves' disease, autoantibodies formation against thyrotropin receptor results in excessive thyroid hormone secretion and goiter.<sup>5,6</sup>

Propylthiouracil and Methimazole/Carbimazole are the anti-thyroid drugs used for the treatment of hyperthyroidism in pregnancy. Propylthiouracil is an anti-thyroid thioureyline drug, belonging to the family of thionamides. Propylthiouracil is presently considered the safest drug to treat hyperthyroidism during early pregnancy.<sup>7</sup>

Conventionally, propylthiouracil has been preferred over Methimazole because of its supposed lower trans-placental passage.<sup>8</sup> Because a limited number of documented research reports of fetal anomalies are related with antenatal use of propylthiouracil, it has been presumed to have a more favorable teratogenic profile than Methimazole.<sup>2,9</sup> But propylthiouracil's antenatal use has been associated with birth defects, though small, since its invention.<sup>10-12</sup> Thus, Propylthiouracil should be used in the first trimester of pregnancy and later changed to Methimazole in the second and third trimester to reduce maternal and fetal risks.<sup>3,13-15</sup>

## MATERIAL AND METHODS

After obtaining prior ethical approval from the Institute Animal Ethical Committee, the study was conducted in

the Teratology laboratory of the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh.

The present study studied<sup>21</sup> adult female Swiss albino mice with an average body weight of 20–25 gm. Under optimal conditions for breeding, Swiss albinomice were housed individually in a departmental animal house. To facilitate mating, female mice were shifted to the cages containing male mice of the same stock in the evening (2 males per female). The presence of vaginal plug marks the 'Day 0' of pregnancy. The pregnant dams were assigned two separate groups, one 'control' and another 'treated.'

**Dose calculation:** The average weight of mice was 20 gm and the average dose of propylthiouracil worked out per mice was 100 mg/kg body weight/day (higher doses were lethal to mice). The stock solution of propylthiouracil was prepared under sterile conditions by pounding the drug (50 mg tablet, available commercially) and dissolving it in 5 mL of distilled water. So, each mL of stock solution contains 10 mg of the drug. Therefore, the amount of stock solution calculated per mice was 0.2 mL containing 2 mg of propylthiouracil. The treated group were given 0.2 mL of the stock solution orally while the control mice were given 0.2 mL distilled water (orally) on 6, 7 and 8<sup>th</sup> day of gestation (period of organogenesis).

The pregnancy was terminated by sacrificing dams near term by cervical dislocation. A midline laparotomy was done and the fetuses were collected. The fetal brains were dissected from both groups and examined for gross malformations. The fetal brains were further processed for microscopic examination with H and E staining.

## RESULTS

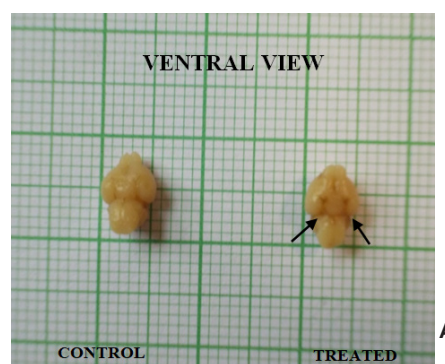
The gross examination of treated brain showed haemorrhagic areas on ventral and dorsal surfaces (Figure 1A, B). The microscopic examination of control brain, under low magnification showed the normal appearance of choroid plexus and ependymal lining of the lateral ventricle (Figure 2A) as compared to their corresponding treated brain showing degeneration of choroid plexus and destruction of ependymal lining of the lateral ventricle (Figure 2 B). Under low magnification, control brain showed the normal appearance of ventricle and hippocampal area (Figure 2C) while dilated ventricle, degeneration of choroid plexus and destruction of hippocampal area were observed in the corresponding treated brain (Figure 2 D). On similar magnification, the control group showed normal fourth ventricle and choroid plexus (Figure 2 E), while corresponding treated brain showed dilated fourth ventricle and degeneration of choroid plexus (Figure 2 F). On higher magnification, the control brain showed normal appearance of choroid

plexus and the ependymal lining (Figure 2G), while degeneration of choroid plexus along with destruction of ependymal lining of the ventricle in the corresponding treated group has been observed (Figure 2H).

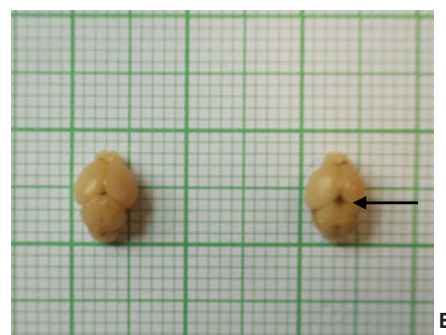
## DISCUSSION

Propylthiouracil is a thionamide derived anti-thyroid drug which is given in the treatment of hyperthyroidism, usually in first trimester of pregnancy. The trans-placental passage of propylthiouracil is adequate enough to cause considerable fetal exposure and its consequent effect.<sup>8</sup> Few documented reports were available on gross and microscopic features on fetal organs (animal and human studies) regarding the teratogenic effects of propylthiouracil.

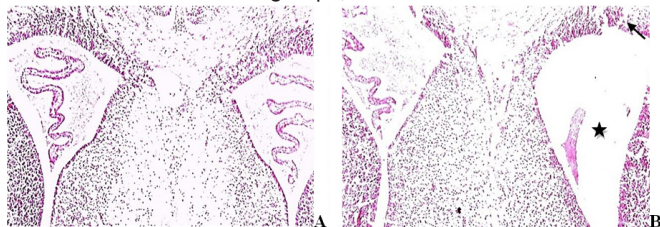
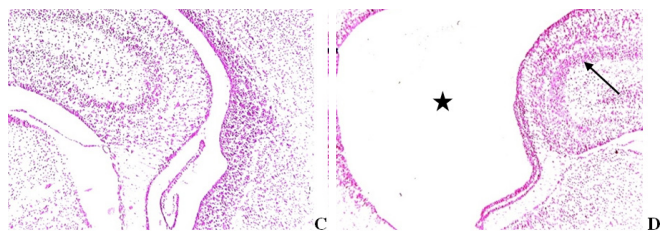
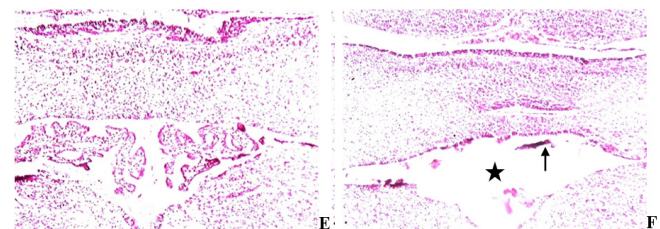
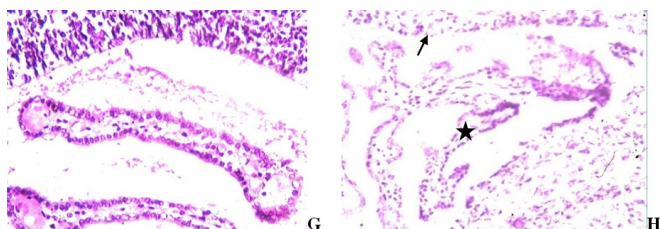
The present study showed that the propylthiouracil exposure during period of organogenesis was associated with gross hemorrhagic areas and microscopic degenerative changes in fetal brain. Mallela et al.<sup>16</sup> observed subcutaneous hemorrhages on the trunk and leg along with focal hemorrhages at the umbilicus and urinary bladder in mice fetuses. In the same study, blood was found in various cavities like nasal, oral, abdominal and pleural cavities.



**Fig 1:** Gross photographs of brain in control and treated groups  
A. Showing ventral surface of control and treated brain. The treated group shows hemorrhagic areas(←)as compared to the control.



**Fig 1: B.** Showing dorsal surface of control and treated brain. The treated group shows hemorrhagic area (←) as compared to the control.

**Figure 2:** Photomicrographs of brain in control and treated groups, H&E**A:** Control brain showing the normal appearance of choroid plexus and ependymal lining of the lateral ventricle; low magnification. **B:** Treated brain showing the degenerated choroid plexus (★) and destruction of ependymal lining of the lateral ventricle (←); low magnification.**C:** Control brain showing the normal appearance of ventricle and hippocampal area; low magnification **D:** Treated brain showing the dilated ventricle (★) and degeneration and destruction of hippocampal area (←); low magnification**E:** Control brain showing the normal fourth ventricle and choroid plexus; low magnification. **F:** Treated brain showing the dilated fourth ventricle (★) and degeneration of choroid plexus (←); low magnification.**G:** Control brain showing the normal appearance of choroid plexus and the ependymal lining; high magnification. **H:** Treated brain showing the degenerated choroid plexus (★) and destruction of the ependymal lining of the ventricle (←); high magnification.

The gross malformations of head and neck region like anencephaly, cleft lip/palate, aplasia cutis congenital and preauricular cyst/sinus has been reported by many workers<sup>17-20</sup> in children born to women with Propylthiouracil exposure during pregnancy but no such abnormalities were observed in the present study, similar to the findings of Korelitz *et al.*<sup>21</sup>

In a previous study,<sup>22</sup> histopathological changes were observed in rat hypothalamus after Propylthiouracil-induced hypothyroidism. There was diffuse vacuolar degeneration and damaged neurons, especially in suprachiasmatic and supraoptic nuclei. The histological features of brain in the present study showed destruction and clumping of degenerated neurons in the hippocampal area, dilatation of the ventricles with destruction of its ependymal lining and degeneration of choroid plexuses.

Yu *et al.*<sup>23</sup> proposed that propylthiouracil was associated with ANCA (anti-neutrophil cytoplasmic antibody) positive vasculitis and the presence of AECA (anti-endothelial cell antibodies). Endothelial damage is an important phenomenon in the early course of vasculitis and there was increasing evidence that AECA could induce endothelium damage by antibody-dependent cellular cytotoxicity. The damage of endothelium may cause leakage of blood into intercellular spaces. This may be a reason for the haemorrhagic areas found in the present study.

Propylthiouracil causes altered differential gene expression involving cytoskeletal remodeling, which defines cell cytostructure and cytodynamics. There was altered cytoskeleton remodeling and keratin signaling pathway. These pathway components play a role in cytoskeletal organization, epithelium morphogenesis and neuronal survival.<sup>7</sup> Yu *et al.*<sup>24</sup> classified Cat. D drugs into IV classes based on their differing effects on primitive streak formation, propylthiouracil belongs to Class II according to this classification. Defective primitive streak formation leads to defective formation of all the three germ layers and structures derived from them.

In vitro studies,<sup>25</sup> has shown that propylthiouracil can be metabolised into different intermediate metabolites namely propyluracil-2-sulfonate (PTU SO<sup>3-</sup>) by both thyroperoxidase and myeloperoxidase (MPO/H<sub>2</sub>O<sub>2</sub>/Cl-system) of phagocytic cells i.e. neutrophilic granulocytes, monocytes and young macrophages. It is a highly reactive compound that covalently binds to sulfhydryl groups of proteins to form sulfide adducts. It has been suggested that this metabolite is responsible for the adverse immunological side effects of propylthiouracil.

## CONCLUSION

The present animal study stated the teratogenic potential of propylthiouracil in developing fetus based on its neurotoxic effect when used in high doses during period of organogenesis. Propylthiouracil cannot be completely avoided as a treatment option in pregnant women with hyperthyroidism, though it is associated with increased risk of fetal malformations, as hyperthyroidism during pregnancy itself is associated with poor maternal and fetal outcome. So, pregnancy-associated

hyperthyroidism must be treated with minimal effective dose of propylthiouracil.

Considering the result from present animal study, thoughtful monitoring and reporting of any adverse effect on mother or fetus/newborn also becomes obligatory for this drug. This study also necessitates for the search of a newer and safer treatment modality for the treatment of pregnancy-associated hyperthyroidism.

### Conflict of Interest

Nil

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Nil

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