# Histopathological Studies of γ HCH induced toxicity on Brain and protective role of *Camellia sinensis* in albino rats

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## Abstract

γ HCH (γ Hexachlorocyclohexane or Lindane) is an organochlorine insecticide being used extensively as broad spectrum insecticide, therapeutic scabicide, pediculocide and ectoparasiticide in human and veterinary medicine. Present study was aimed to investigate the neurotoxic effects of γ HCH and its amelioration by *Camellia sinensis* on histopathological studies in brain of albino rats. Four groups of rats with 18 each were maintained under standard laboratory hygienic conditions and provided feed and water *ad libitum*. γ-HCH was gavaged @ 20 mg/kg bwt using olive oil as vehicle to groups II. *Camellia sinensis* @100 mg /kg body wt was administered orally in distilled water to group IV in addition to γHCH 20mg/kg bwt up to 45 days to study ameliorative effects. Group I and III were treated with distilled water and *Camellia sinensis* (100 mg /kg body wt) respectively. In group II, the cerebral cortex revealed congested choroid plexus, mild capillary proliferation, satelitosis, shrunken neurons and perineuronal vacuolation. In cerebellum perineuronal vaculation, rounding of purkinje cells were observed. The sections of group IV revealed mild congestion and mild degeneration of purkinje cells in cerebellum, whereas group III animals did not reveal any significant histopathological changes. In conclusion, these results suggest that exposure to γ HCH (100 mg/kg) in male rats induced histological alterations in brain, and co administration of *Camellia sinensis* brought moderate protection accordingly.

**Key words**: Brain; γ HCH; albino rats; *Camellia sinensis*.

# Introduction

Pesticides are used in every realm of the environment to control pests, such as insects, weeds, fungus and rodents. An estimated 85-90% of pesticides never reach their target organisms (Repetto and Baliga, 1996) and it is very likely that many non-target organisms are exposed to multiple pesticides throughout their lifetimes, either sequentially or concurrently. Technical grade Hexachlorocyclohexane consists of a mixture of isomers (16% α, 7% β and 45% γ isomers) produced by photo chlorination of benzene. Gamma HCH production involves purification of technical grade HCH, only the γ isomer of HCH has broad spectrum insecticidal property. It is available as suspension, emulsifiable concentrate and used as fumigant, seed treatment, wettable and dustable powder and ultra volume liquid (UVL) (Banerjee *et al*., 1999). γ HCH (Lindane) causes neurological effects in animals which include enhanced synaptic activity (Joy, 1982), altered GABA functional activity (Joy and Albertson, 1985), and inhibition of GABA (Nakajima, 1983) or oxidative damage to $Na^+$-$K^+$-ATPase activity (Sahoo and Chainy, 1998). In general, the mechanism of toxicity of γ HCH on the nervous system appears to be similar to those of other neurotoxic organochlorine insecticides (Bano and Bhatt, 2007).

In fact, the herbal medicines derived from plant extracts are being increasingly utilized as adjunct treatment options for a wide variety of clinical disease (Nandakumar *et al*., 2008). More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities (Frei and Higdon 2003). *Camellia sinensis* is commonly known as green tea. As the tea is one of the most widely consumed beverages in the world, second to water, its medicinal properties have been widely explored. The green tea contains the polyphenols, like gallic acid and catechin, and their derivatives like theogallin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (EGCG) (Guo *et al*., 1996). Green tea polyphenols have demonstrated significant antioxidant properties (Zhao *et al*., 2001)

Though wide information is available on mechanism of neurotoxicity of γ HCH but the reports on its histopathology are scanty. Therefore, a study has been made to evaluate the histological alterations induced by γ HCH and its amelioration by *Camellia sinensis* in the brain of male rats. In the present study, the cerebral cortex, cerebellum and medulla were considered as these regions are critically involved in the co-ordination and maintenance of the body.

# Materials and methods

**Test chemical**: γ- HCH was purchased from Sigma Aldrich Pvt. Ltd., Bangalore, India. The *Camellia sinensis* (green tea) whole leaf extract powder with product code No. P/SVU/CASI-01 was procured from Chemiloids Company, Vijayawada, Andhra Pradesh state.

**Animal model:** Male Wistar Albino rat were selected as experimental animals for the present study. Rats of 2 -3 months, weighing 150±30 grams were housed four per cage with free access for food and water *ad libitum*. The rats were maintained in the animal house of Department of veterinary pathology, College of Veterinary Science, Tirupati, at 25±1°C with a day night cycle of 12 hours under hygienic conditions providing the standard laboratory feed. All animal experiments were carried out as per the guidelines of Institutional Ethical Committee.

**Experimental Design:** The rats were divided into four groups of 18 rats in each. Group I served as control (0.3 ml of water by oral administration), Group II rats were treated with lindane (20 mg/kg bwt by oral administration), Group III rats were treated with Green tea (100 mg/kg bwt dissolved in water by oral administration) and Group IV rats were co-treated with lindane (20 mg/kg bwt) and Green tea (100 mg/kg bwt), First the animals were treated with lindane followed by green tea after 15 min. Six rats from each group were randomly sacrificed every fortnight i.e. on the $15^{th}$, $30^{th}$ and $45^{th}$ day. Rats were anesthetized by exposing to diethyl ether and simultaneously sacrificed by cervical decapitation.

**Histopathology:** Cerebellum and cerebral cortex were collected from the control and experimental animals and gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and debris adhering to the tissues. Histopathological examination of the brain tissues was followed as per Culling (1974). Tissues were fixed in 5% formalin for 24 hours. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohol, the tissues were cleared in xylene, embedded in paraffin wax. Sections were cut at 6 μ thickness and stained with hematoxylin and eosin (H&E), mounted in DPX mount and observed under microscope.

## Results

All the rats in γ-HCH treated group (Group II) showed convulsions and seizures 15 to 30 min after feeding the γ-HCH and other nervous symptoms like hyper irritability and scratching of face. Throughout the 45 days of experiment the green tea ameliorated group (Group IV) was apparently normal without any clinical signs.

Grossly there was no significant changes in brain of rats during the 15$^{th}$, 30$^{th}$ and 45$^{th}$ day of the experimental period in both γ HCH treated group and ameliorated group.

Microscopically by the 15$^{th}$ day of γ-HCH treatment, the cerebral cortex revealed congested choroid plexus, mild capillary proliferation (Fig. 1), satelitosis (Fig. 2) and shrunken neurons, cerebellum revealed perineuronal vacuolation and demyelinating changes (Fig. 3). Similar lesions like meningeal and choroid blood vessel congestion (Fig. 4), mild satelitosis, neuronophagia with moderate intensity, shrinkage of neurons (Fig. 5) and glial cell proliferation (Fig. 6) in cerebrum, multiple areas of congestion (Fig. 7) and capillary proliferation (Fig. 8) in cerbellum were seen conspicuously in rats brains of 30$^{th}$ day of experiment. At the end of the 45$^{th}$ day of the experiment, congestion of choroid plexus, neuronophagia, shrinkage of neurons, perineuronal vacuolation (Fig. 9), proliferation of astroglial cells and demyelinating change with spongy appearance were prominent in cerebrum. In cerebellum rounding of purkinje cells or ghost appearance of purkinje cells (Fig. 10) were observed. Neuronophagia, mild glial cell proliferation and atrophy of neurons (Fig. 11 & 12) with mild intensity were noticed in green tea treated group by the end of the 15$^{th}$ day and 30$^{th}$ day of experiment. By the end of 45$^{th}$ day of experiment these changes were very mild.

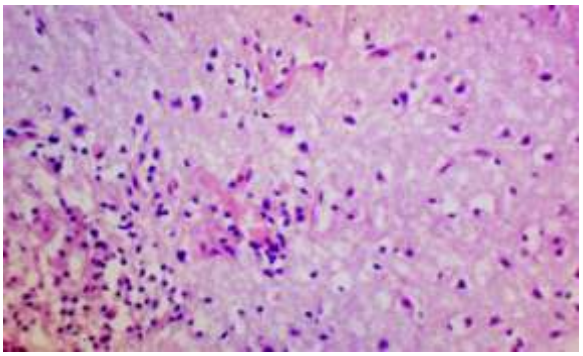

Fig. 1 Mild capillary proliferation in cerebral cortex of γ HCH treated rats. H & E: x .280.

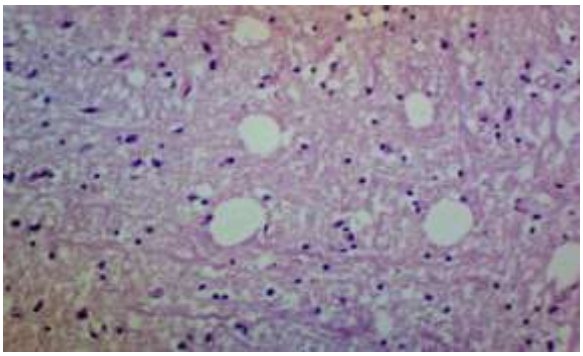

Fig. 2 Section showing satelitosis in cerebral cortex of γ HCH treated rats. H & E: x .280 Fig. 2 Section showing satelitosis in cerebral cortex of γ HCH treated rats. H & E: x .280

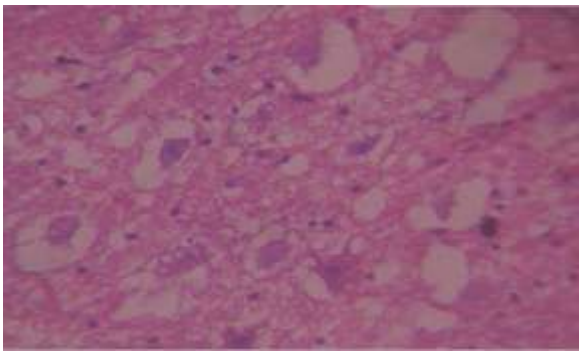

Fig. 3 Perineuronal vacuolation and demyelinating changes in cerebral cortex of γ HCH treated rats. H & E: x .280.

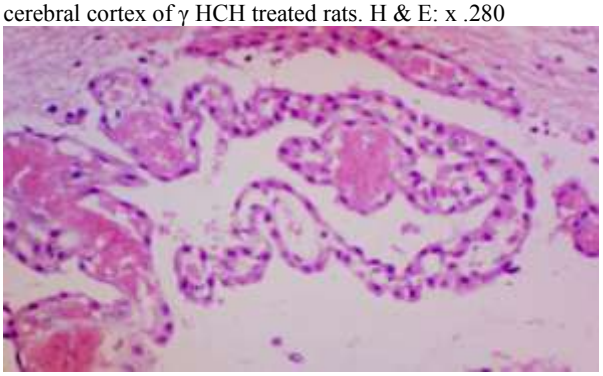

Fig. 4 Section showing choroid blood vessel congestion in cerebral cortex of γ HCH treated rats. H & E: x .280

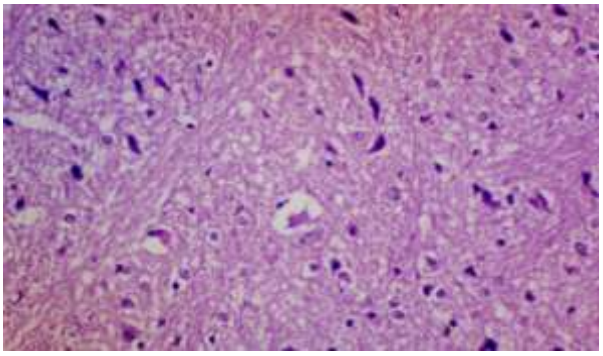

Fig. 5 Section showing shrinkage of neurons in cerebral cortex of γ HCH treated rats. H & E: x .280.

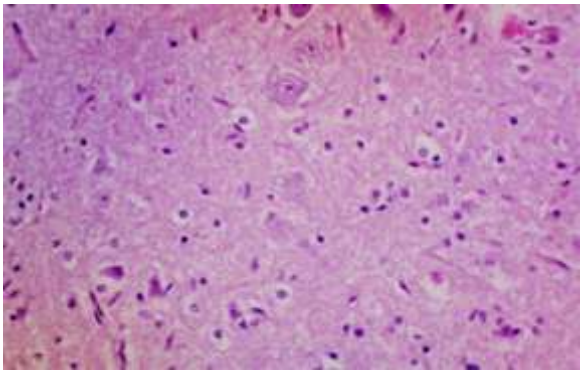

Fig. 6 Section showing glial cell proliferation in cerebral cortex of γ HCH treated rats. H & E: x .280.

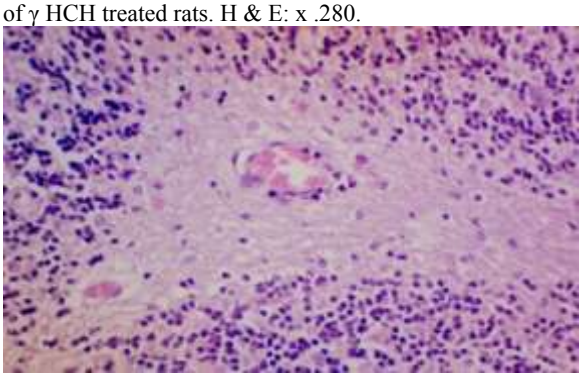

Fig. 7 Section showing multiple areas of capillary haemorrhages in cerebral cortex of γ HCH treated rats. H & E: x .280.

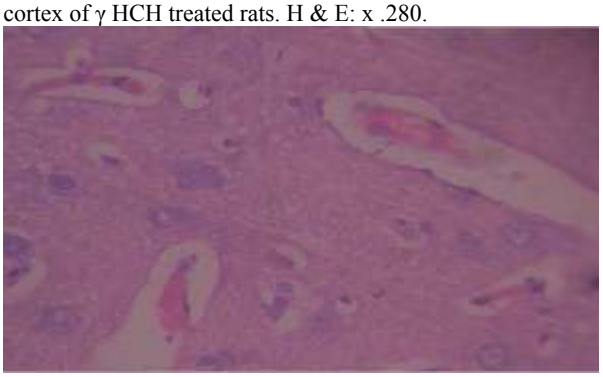

Fig. 8 Section showing capillary proliferation in cerebral cortex of γ HCH treated rats. H & E: x .280.

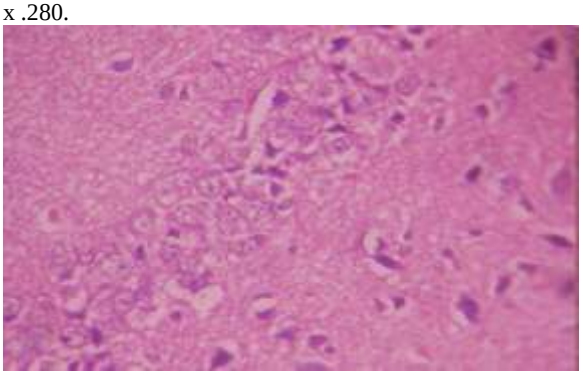

Fig. 9 Section showing perineuronal vacuolation in cerebral cortex of γ HCH treated rats. H & E: x .280.

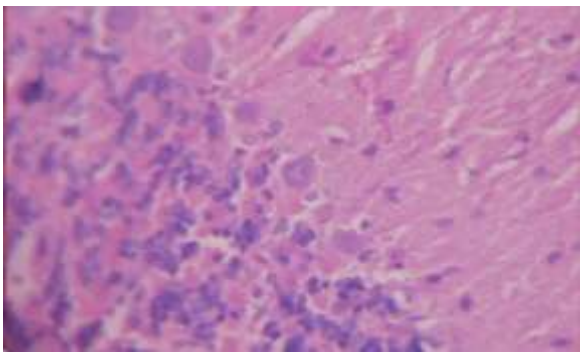

Fig. 10 Section showing rounding appearance of purkinje cells with loss of dendrites in cerebellum of γ HCH treated rats. H & E: x .280.

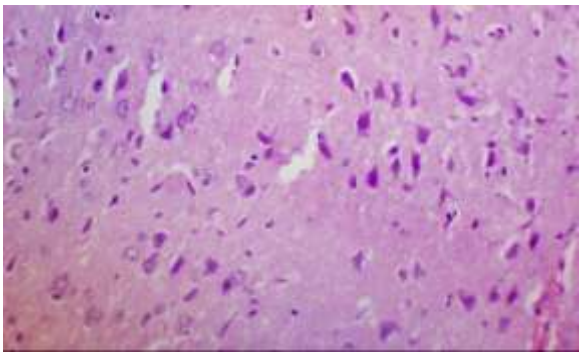

Fig. 11 Section showing mild degenerative changes of neuron in cerebral cortex of Camellia sinensis treated rats. H & E: x .280.

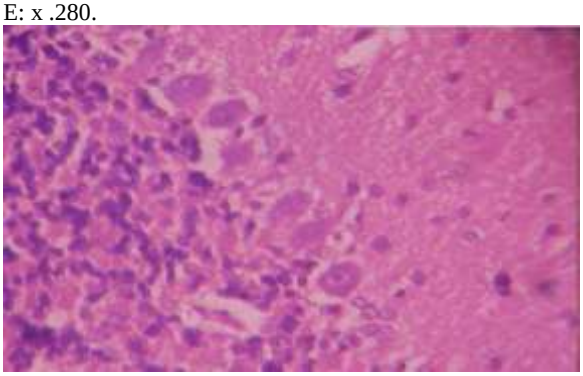

Fig. 12 Section showing mild degenerative changes of purkinje cells in cerebellum of Camellia sinensis treated rats. H & E: x .280.

## Discussion

The present study is related to histopathological changes in the brain of γ HCH treated rats. Severe pathological lesions were observed during 45$^{th}$ day of experiment rather than 15$^{th}$ and 30$^{th}$ day of experiment. Brain sections of 15$^{th}$ day γ-HCH treated rats revealed degeneration of neurons, neuranophagia, chromatolysis, congested cerebral vessels, demyelinating changes in white matter of cerebellum. Similar lesions were seen in brain of 30$^{th}$ day of experiment. At the end of 45$^{th}$ day of experiment congestion of choroid plexus, neuronophagia, shrinkage of neurons, demyelination with spongy appearance of white matter and proliferation of astroglial cells were prominent in cerebrum. In cerebellum, rounding of purkinje cells is conspicuous. Similar findings were reported by Fidan *et al*. (2008) and Papaioannou *et al*. (1988). Brain revealed moderate ameliorative changes in green tea ameliorated group of rats at the end of 45$^{th}$ day. Tariq and Riyaz (2013) reported similar findings.

**Conclusion**

The changes recorded in this study might be due to accumulation of γ HCH and its metabolites in the brain. Although *Camellia sinensis* plays a pivotal role in neutralizing the free radicals, this study revealed mild to moderate amelioration. More detailed studies are still needed to elucidate the ameliorative effects of green tea at higher doses.

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