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# Experimentally Induced Toxicity of Ochratoxin A and Endosulfan in Male Wistar Rats: A Hormonal Disorder

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**Abstract:** Dietary exposures to food contaminants such as mycotoxin (s) or pesticide (s) are most significant due to their adverse effect on the production and reproduction in animals and human population. The present investigation was conducted to evaluate the adverse effects of Ochratoxin A (OTA) and endosulfan on the male hormonal status. OTA (4 mg $kg^{-1}$ feed) and endosulfan (5 mg $kg^{-1}$ body weight) were administered orally alone and in combination for 30 days caused significant alterations on the serum levels of various body hormones such as thyroid hormones (triiodothyronine and thyroxin), testosterone, prolactin, insulin and cortisol of adult male Wistar rats. Radioimmuno assay revealed significantly higher serum levels of Thyroxin ($T_4$) and prolactin and the significantly lower serum levels of Triiodothyronine ($T_3$), testosterone, insulin and cortisol in all the treated groups in comparison to control. Moreover, the hormonal changes were maximum in the combination group. In conclusion, the simultaneous exposure of OTA and endosulfan caused more pronounced hormonal alterations possibly may due to their additive interaction in adult male Wistar rats.

**Key words:** Ochratoxin A, endosulfan, male rats, hormone, radioimmuno assay, India

## INTRODUCTION

Ochratoxin A (OTA), a potent nephrotoxic mycotoxin, mainly produced by several species of fungal genera Aspergillus and Penicillium. It is a widespread natural contaminant in various food or feed commodities, resulting in toxicoses in animal and human populations. OTA possesses general structure of L-β-phenylalanine linked by an amide bond to dihydro-isocoumarin moiety. Unfavourable elimination toxicokinetics of OTA displays an extremely high inter-species variability and has been found to exert its deleterious effects in terms of nephrotoxicity, mutagenicity, neurotoxicity, immunotoxicity, carcinogenicity, genotoxicity and teratogenicity in various mammalian species (Dietrich *et al*., 2005; Pfohl-Leszkowicz and Manderville, 2007; Amezqueta *et al*., 2009; Duarte *et al*., 2010). OTA has been suggested by various researchers to mediate its toxic effects via induction of apoptosis, disruption of mitochondrial respiration and/or the cytoskeleton or via generation of DNA adducts (Brien and Dietrich, 2005). Studies have also shown the presence of OTA in humans serum, breast milk and kidneys suggesting its public health significance (Petzinger and Ziegler, 2000). Endosulfan, an organochlorine insecticide that belongs to the cyclodiene group is one of the most commonly used pesticides to control pests in vegetables, cotton and fruits in the agriculture field. It has been classified by WHO (2002) in the category of technical products that are moderately hazardous (class II) for various species of animals and human beings. It is a potential environmental pollutant and has gained public health significance (USEPA, 1980) due to its low water solubility, chemical stability, high lipid solubility and slow rate of biotransformation there is bioconcentration and biomagnifications of endosulfan in food chains by its lipophilic nature. In India, endosulfan residues have been reported to occur in high levels in

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various samples of cashew, fruits, milk, butter, coconut oil, soil, ground water and even in human blood in Kasargod district of Kerala (Jayashree and Vasudevan, 2007). It has also been studied experimentally for its endocrine-disrupting potential (Bisson and Hontela, 2002; Hiremath and Kaliwall, 2002) and also reported to cause hepatic tumor (Fransson-Steen *et al*., 1992), apoptosis (Kannan *et al*., 2000), oxidative stress (Singh and Pandey, 1990) and immunotoxicity. In human, it is also reported to cause social death (Chugh *et al*., 1998), uterine leiomyomas, non-Hodgkin's lymphomas (Zahm and Blair, 1992; Zahm *et al*., 1993) and delayed sexual maturity due to interference with sex hormone synthesis (Saiyed *et al*., 2003). It targets the prefrontal cortex of the brain (Cabaleiro *et al*., 2008) and may be implicated in Parkinson's disease (Wang *et al*., 2006) and also caused adverse behavioural effects (ATSDR, 2000). Exposure in utero causes significant teratogenic effects in pregnant rats (Singh *et al*., 2006). The perused literature revealed very scanty information on the toxicity of OTA and endosulfan for their adverse effects on the body hormones in adult animals or human being. Moreover, no report could be traced in the literature on the combined effect of these two commonly occurring environmental pollutants (OTA and endosulfan) in animals or human being. The present research was therefore, designed to study the effects of OTA and endosulfan alone and in combination in male adult Wistar rats exposed for 30 days.

## MATERIALS AND METHODS

**Production and analysis of OTA:** *Aspergillus ochraceus* NRRL-3174 was originally procured from National Centre for Agriculture Utilization Research (NCAUR-3174) Peoria, Illinois, USA. It was grown on sterilize maize as per the method described by Trenk. The extraction and clean up of the toxin sample were done as per the method of AOAC (1995) and the quantitative determination of the toxin was done by using thin layer chromatography method and TLC scanner (CAMAG, Switzerland).

**Preparation of toxicated feed:** Cultured maize powdered containing known amount of OTA was added to basal ration (which were tested negative for presence of contaminating mycotoxins) in such proportion that the final concentration of OTA was adjusted to 4 mg $kg^{-1}$ feed. Aliquots were taken from the mixed diet and the toxin was further quantified to ensure the proper mixing of the toxin.

**Dosing of endosulfan:** Technical grade (>99.98% pure crystalline form) endosulfan was procured from Shriram Chemicals Ltd., India was dissolved in corn oil (vehicle) and orally intubated to male rats at the rate of 5 mg $kg^{-1}$ body weight, daily for 30 days.

The treatment volume was 0.1 mL/100 g of body weight. Fresh solution of endosulfan was prepared on each day of treatment. Control animals received an equal volume of corn oil similar to those treated with endosulfan.

**Experimental animals and design:** Forty sexually mature (180±20 g), male Wistar rats, procured from the Laboratory Animal Resource (LAR) section of the Indian Veterinary Research Institute were maintained on standard feed and water available *ad libitum*. After a 1 week of acclimatization period, the animals were randomly distributed into IV groups of ten rats in each and treated for 30 days as follows: group I animals was given with a diet containing OTA at the level of 4 mg $kg^{-1}$ feed; group II animals received endosulfan at the rate of 5 mg $kg^{-1}$ body weight by oral intubation; group III animals received OTA (4 mg $kg^{-1}$ feed) and endosulfan (5 mg $kg^{-1}$ body weight) and finally and group IV animals were fed a standard mycotoxin free basal diet. All the experimental procedure and sacrifice of rats were carried out as per the approved guidelines of Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Hormonal estimations:** The blood sample was collected from the heart of the animal at time of sacrifice. The blood samples (1.5-2.0 mL) was collected in dry, clean sterilized test tubes and allowed to clot. The sera samples were further analysed for hormonal estimations of Triiodothyronin ($T_3$) and Thyroxin ($T_4$), testosterone, insulin, prolactin and cortisol using Radioimmuno Assay (RIA) kits supplied by Immunotech, France using auto-gamma counter (Packard Bio Science Company Model Cobra II, USA).

These hormones were estimated in serial dilutions of serum samples along with parallel curves to standards. All the sera samples for hormone estimations were processed in one assay to rule out inter-assay variations.

**Statistical analysis:** Data generated during the study were suitably analysed using one way Analysis of Variance (ANOVA) to detect differences among groups and the means were compared by Dunnett's multiple comparison test. All analyses were performed with GraphPad InStat software (San Diago, USA). All the statements of significance were based on a probability level of $p<0.01$.

## RESULTS AND DISCUSSION

In the present study, the Wistar rats were chosen because of their genetic stability, practical convenience in housing and maintenance and low cost. Moreover, the rat is a suitable laboratory model recommended for the various toxicological studies by the regulatory authorities. Since, the exposure of environmental pollutants such as mycotoxins or pesticides to animals and human populations occurs mostly through the contaminated diet and the oral route which was employed in the present study simulate the more natural and realistic route of toxicity due to OTA and endosulfan. The effects of OTA and endosulfan alone and in combination on hormonal status of adult male Wistar rats are shown in Table 1.

Table 1: Effects of ochratoxin A and endosulfan on various body hormones of adult male Wistar rats

| Name of hormones | Groups I | II | III | IV |
|---|---|---|---|---|
| Triiodothyronine (T3); ng moL$^{-1}$ | 2.82±0.11$^{b}$ | 3.49±0.11$^{b}$ | 2.54±0.08$^{c}$ | 4.17±0.15$^{a}$ |
| Change (%) | (-) 32.27 | (-) 16.31 | (-) 39.09 | - |
| Thyroxin (T4); ng moL$^{-1}$ | 95.86±4.80$^{b}$ | 98.07±5.27$^{b}$ | 113.29±6.04$^{c}$ | 84.46±4.57$^{a}$ |
| Change (%) | (+) 13.5 | (+) 16.1 | (+) 34.1 | - |
| Testosterone; ng moL$^{-1}$ | 1.47±0.08c$^{b}$ | 1.65±0.07$^{b}$ | 1.37±0.06$^{c}$ | 1.97±0.04$^{a}$ |
| Change (%) | (-) 25.4 | (-) 16.2 | (-) 30.5 | - |
| Insulin; IU mL$^{-1}$ | 0.026±0.002$^{b}$ | 0.027±0.094$^{b}$ | 0.018±0.002$^{b}$ | 0.048±0.003$^{a}$ |
| Change (%) | (-) 45.8 | (-) 43.8 | (-) 62.5 | - |
| Prolactin; ng moL$^{-1}$ | 1.74±0.087$^{b}$ | 1.62±0.071$^{b}$ | 2.29±0.113$^{c}$ | 1.30±0.037$^{a}$ |
| Change (%) | (+) 33.9 | (+) 24.6 | (+) 76.2 | - |
| Cortisol; μg dL$^{-1}$ | 153.23±4.97$^{b}$ | 158.23±4.58$^{b}$ | 123.95±3$^{c}$ | 176.45±2.58$^{a}$ |
| Change (%) | (-) 13.2 | (-) 10.3 | (-) 29.8 | - |

Group-I, ochratoxin A (4 ppm in feed); group-II, endosulfan (5 mg kg$^{-1}$ body weight); Group-III, ochratoxin A+endosulfan (4 ppm in feed+5 mg kg$^{-1}$ body weight); Group-IV, healthy control; values are expressed as Mean±SE (n = 10); mean bearing at least one common superscript do not vary significantly between groups ($p \leq 0.01$)

**Thyroid hormones:** A significant higher serum levels of Thyroxin ($T_4$) whereas significantly lower serum levels of Triidothyronin ($T_3$) in all the treated groups. The serum levels of $T_3$ might have led to some effect on testicular Sertoli and Leydig cells which ultimately resulted in decreased synthesis and secretion of testosterone. Similar observations have also been made by earlier researchers (Maran *et al*., 2000) in experimental hypothyroid adult rats. It is seen that after isolating Leydig cell from hypothyroid adult rats. It is seen that after isolating Leydig cells from hypothyroid adult rats resulted in reduced testosterone, secretion. Sinha *et al*. (1991) reported increased serum levels of $T_4$ and decreased serum levels of $T_3$ in female freshwater catfish exposed with endosulfan for 96 h to 16 days. Thyroid hormones are essentially required for proper nerve cell proliferation, cell migration and differentiation in developing mammalian brain (Porterfield and Hendry, 1998). Schantz and Widholm (2001) also studied the adverse effect of organochlorine pesticides on thyroid hormones in rats and found the reduced serum levels of $T_4$ however, the levels of active form of hormone $T_3$ were mostly unchanged or only slightly reduced. The finding on thyroid hormone profile is in agreement with observations made by Paul *et al*. (1992, 1994, 1995) in rats. Leydig cells are primary source of androgen hormones in mammalian males. Thyroid hormones, $T_4$ and $T_3$ have some role in proper functioning of Leydig cells. $T_3$ is 4.5 time more potent than $T_4$. Palmero *et al*. (1990) demonstrated a specific binding site of thyroid hormone to nuclei of Sertoli cell in rats. Luo *et al*. (1988) also demonstrated thyroid receptor protein in testicular interstitial tissue of adult rats. Hardy *et al*. (1996) have reported the presence of thyroid hormones receptor in Leydig cell and their precursors in rats of different ages including adult animals.

**Testosterone:** In this study, OTA, endosulfan and their combination caused significant reduction in serum levels of testosterone. Information regarding the effects of endosulfan and OTA on testosterone levels are scanty. However, Singh and Pandey (1990) also reported profound decreased levels of plasma testosterone associated with decrease in testicular testosterone in pubertal rats exposed to endosulfan for 30 days. Various experimental studies in male adult rats suggested that endosulfan can affect the male reproductive system by reduced intratesticular spermatid counts, sperm abnormalities and changes in the marker enzymes of testicular activities and these effects are likely to be greater if exposure occurs during the developmental phase. Moreover, it depresses testosterone levels and may cause reproductive toxicity in humans (ATSDR, 2000) and further suggested that endosulfan exposure in male children may delay sexual maturity and interfere with sex hormone synthesis (Saiyed *et al*., 2003). It also interferes with the steady state levels of oestrogen causing proliferation of MCF-7 human breast cancer cells and its effects on the endocrine system indicate that endosulfan is likely to cause the onset and/or development of mammary tumours (Je *et al*., 2005; Chatterjee *et al*., 2008).

**Insulin:** Endosulfan and OTA are known to cause increased blood glucose level. Insufficiency of insulin hormones lead to increased in blood glucose level. The oxidative stress induced damage to beta cell of Langerhans could have resulted in hypoinsulimeia in the rats. Increased blood glucose levels and ultrastructural

changes like vacuolation, swelling of mitochondria and pycnotic nucleus in beta cells of Langerhans after oral endosulfan administration have also been reported earlier in rats (Naqvi and Vaishnavi, 1993; Kalender *et al*., 2004). These changes were indicative of irreversible damage. Biochemical studies showed that endosulfan affect integral protein and receptors on cell membrane (Kiran and Varma, 1988). Garg *et al*. (1980) reported the increased blood glucose levels after administration of endosulfan orally in rats. Hagar *et al*. (2002) observed the increased levels of blood glucose and decreased levels of insulin after dimethoate administration which is an organophosphorus insecticide. Moreover, Subramanian *et al*. (1989) have demonstrated the diabetogenic nature of OTA in rats exposed with 100 μg $kg^{-1}$ feed for 8 weeks which resulted in increased blood glucose and decreased serum levels of insulin.

**Cortisol:** In present investigation, treatment of rats with OTA, endosulfan and their combination showed significantly reduced serum levels of cortisol. The *in vitro* inhibition of cortisol secretion by endosulfan exposure in adernocortical cell (Dorval *et al*., 2003) and in kidney cell (Leblond *et al*., 2001) of rainbow trout further support the present *in vivo* findings in rats as well. The chronic exposure to agrochemical may affect endocrine and metabolic functions as well as activity of enzyme that chronic exposure of fish to various associated with oxidative stress. Adrenocortical tissue has a high content of pesticides or heavy metals may interact with catalytic cycle of cytochrome P-450's and disturb the cycle to induce formation of reactive oxygen species. Interaction of endosulfan with cytochrome P-450's mediating cortisol synthesis may then be responsible by induction of oxidative stress for loss of activity of sterroidogenic acute regulatory protein expression (Walsh and Stocco, 2000). This protein is involved in transfer of cholesterol of inner mitochondrial membrane where cytochrome P-450's side chain cleavage enzyme initiates the synthesis of steroid hormones. Endosulfan may distrust steroidogenesis through similar mechanism. The information regarding effect of OTA on cortisol secretion is lacking. However, the results of this study show that OTA may affect the steroid hormone by similar mechanism to that of endosulfan and hence might be responsible for positive correlation in the simultaneous exposure in male adult rats.

**Prolactin:** It is secreted by the anterior pituitary gland, play a vital role in physiological growth of testes, adrenals and accessory sex glands. The serum levels of prolactin were found to be increased in all the treatment groups. The higher serum values of prolactin might possibly be due to signal sent to adenohypophysis in presence of low serum levels of serum testosterone in rats. Due to this feedback signal, adenohypophysis secreted more prolactin as a positive response to act on testes. Due to paucity of literature on the effects of endosulfan and OTA on pituitary gland there is need to address these deficiencies to property understanding and to appreciate the function of endocrine glands particularly thyroid and pituitary and ultimately their effects on the male reproductive system. The adverse effects of OTA and endosulfan on the serum levels of various body hormones in adult male Wistar rats are more severe in combination treatment. There appears to be no published report on the combined effect of OTA and endosulfan in adult male rats though both of these have been found to be co-contaminants of feed samples under field conditions.

## CONCLUSION

The present study confirms that the individual and combined exposure of OTA at 4 mg $kg^{-1}$ feed and endosulfan intubated orally at the rate of 5 mg $kg^{-1}$ body weight for 30 days caused significant alteration on hormonal status in adult male Wistar rats and the hormonal changes are more severe in the combination group, strongly suggesting their additive interaction.

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