Lindane Induces Spermatotoxicity and inhibits Steroidogenesis in Adult Rats


Abstract
This study was conducted to investigate the influence of lindane on spermatogenesis and testicular biochemical parameters and to get insight into its mechanism. Adult male albino rats were treated orally with lindane at doses of 0, 1, 2 or 4 mg/kg/day for 30 consecutive days. Testes weight and sperm count and motility were significantly decreased. Testicular activities of 3β-HSD, 17β-HSD were significantly inhibited. Testicular cholesterol level was significantly increased while glycogen and sialic acid content were significantly decreased. Testicular activities of LDH-X, γ-GT, β-glucuronidase and acid phosphatase (ACP) were significantly decreased. Aldo-ketoreductase activity was significantly decreased in response to 2 or 4 mg/kg/day of lindane while, protein carbonyl contents were significantly increased. Hydrogen peroxide and hydroxyl radical generation and LPO were significantly increased. The enzymatic antioxidants SOD, CAT and GPx and the non-enzymatic antioxidants GSH and Vit C were significantly decreased. Lindane at doses 2 and 4 mg/kg/day showed a significant reduction in Vit E, while at a dose of 1 mg/kg/day did not show any significant change. In conclusion, lindane inhibits spermatogenesis, steroidogenesis and suppresses Sertoli cell marker enzymes which may be due, partly, to oxidative stress. Lindane enhances ROS generation and LPO and depletes antioxidant enzymes and non-enzymatic antioxidants.

Keywords
Lindane; Testis; Spermatogenesis; Steroidogenesis; Oxidative stress

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Introduction
Exposure to toxins during spermatogenesis may target male germ cells that result in abnormal functioning and adverse pregnancy outcomes [1]. In the past few years, there has been increased interest in assessing the relationship between impaired male fertility and environmental factors [2,3]. Lindane, the 6-isomer of hexachlorocyclohexane (also known as -HCH), is an organ chlorinated insecticide, widely used in agriculture and as a therapeutic scabicide, pediculicide, and ectoparasiticide for humans and animals [4]. The extensive use, chemical stability and bioaccumulation potential of lindane have resulted in its ubiquitous distribution in the ecosystem and thereby considered to be a global pollutant [5]. Several studies have demonstrated the repro-toxic effects of lindane in rodents and in fact, male gonads have been found to be highly sensitive target organs for lindane [6]. Degenerative changes such as tubular atrophy, necrosed spermatogenic cells, and...
enlargement of the interstitial space have been observed in adult rat testis following oral (17.6 mg/kg for 90 days) administration of lindane [7]. Other reported reproductive effects of lindane include alteration in activities of ATPase of testicular plasma membrane [8], impaired spermatogenesis [9] and steroidogenesis [10] decreased sperm count [11] and increased sperm abnormalities [12], changes in testosterone metabolism and plasma testosterone levels [12,13], and alterations in Leydig and Sertoli cells [6,14]. All of these effects impair male reproductive function. Lindane has been reported to induce oxidative stress by interacting with the cell membrane, triggering the generation of ROS and altering the level of antioxidant molecules which in turn cause severe physiological dysfunction in various organs such as liver, testes and brain [15,16]. Alterations in the levels of heat shock protein and clusterin accompanied by an induction of stress in rat testis as early as 12 h following exposure to lindane were also reported [17]. Transient inhibitory effect of lindane on testicular steroidogenesis and the possible role of hydrogen peroxide (H2O2) in mediating these effects were demonstrated by Saradha [18]. Other studies clearly demonstrated the detrimental effects of lindane on testicular functions and its strong association with oxidative stress and reactive oxygen species (ROS) [19]. The stress response and programmed cell death are cellular reactions to stressful stimuli. Stress-induced apoptotic alterations have been implicated as a cause or consequence of various pathological states including infertility [20,21]. Still through studies on testicular toxicity by lindane are lacking which needs attention in greater detail. This study was conducted to further investigate the influence of lindane on spermatogenesis and testicular biochemical parameters in order to assess the male reproductive toxicity of lindane and to get more insight into its mechanism.

**Materials and Methods**

**Chemicals**

Lindane (γ-isomer) (99% pure), pyrogallol, sodium azide, Alexa Fluor-488-PNA, Rh123 and glutathione reductase were purchased from Sigma–Aldrich Chemical Company, St. Louis, MO, USA. All other chemicals are of analytical grade.

**Animals and treatments**

Healthy adult male Wistar rats (90 days) weighing 180-200 g were housed in clean polypropylene cages and maintained on a 12 h light: dark cycle and a temperature of 20-25°C with ad libitum access to food (standard rat chow) and water. All the experiments with animals were carried out according to the guidelines of the Institutional Animal Ethical Committee. For 7 days before the experiment, rats were handled daily for 5 min to acclimatize them to human contact and minimize their physiological responses to handling for subsequent protocols [22]. Lindane was dissolved in olive oil and given to rats by gavage at 0, 1, 2 or 4 mg/kg/day for 30 consecutive days. Gavage volume was adjusted according to the weight of each rat. The doses and duration were selected based on as per