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STEROIDOGENIC INHIBITION IN TESTICULAR TISSUE OF FORMALDEHYDE EXPOSED RATS

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Abstract: Three groups of rats (n=10) were subjected to intraperitoneal treatment of formaldehyde daily at doses of 5, 10 and 15 mg/kg body weight over a period of 30 days. Gradual diminution in body and testicular weight was observed in all treated groups. Leyding cell impairement was conspicuous in those given doses of 10 and 15 mg/kg. Inhibition of 3 β-Δ^5–hydroxy steroid dehydrogenase and accumulation of sudanophillic materials in testicular tissue of formaldehyde treated rats was recorded histochemically. Significant decline of serum testosterone was also observed in the same groups. Structural and functional impairement of Leydig cells after formaldehyde treatment caused steroidogenic inhibition.

Key words: steroidogenesis formaldehyde testis

INTRODUCTION

Industrial use of formaldehyde (HCHO) and its hazards are well documented. General epidemiological data from different developing countries indicate that skin irritation, headache, nausea and vomitting occur in the workers due to short exposure to HCHO when they are at work (1, 2). Carcinogenic potential of formaldehyde in respiratory organs has already been reported in occupationally exposed workers (3). Formaldehyde treatment exhibited mutagenic effects in rodents (4). After chronic exposure to formaldehyde inhibition of cellular DNA was reported, which leads to carcinogenic and mutagenic effects (5, 6). Reports of HCHO effects on reproduction are scanty with only one report on chronic exposure of formaldehyde over a period of 30 days causing spermatogenic inhibition alongwith degeneration of the testicular tissue in rats (7). The present investigation was therefore, undertaken to ascertain whether morphological changes of testicular tissue are also accompanied by the change of steroidogenic process alongwith functional status in the testis after treatment with formaldehyde.

METHODS

Forty male rats of Chalres foster strain weighing 150±5 gm were used for the experiment. Animals were maintained in an airconditioned animal house at temperature 25±2°C on a standard diet and water *ad libitum*. Animals were equaly divided into four groups (n=10). Control group received only distilled water intrapertioneally (i.p.) as vehicle, wheareas other three groups were given formaldehyde at doses of 5, 10 and 15 mg/kg body weight i.p. (10%, 20% and 30% of LD_{50}, 50 mg/kg i.p. in rat) over a period of 30 days (8).

Serum testosterone assay : Next day after completion of treatment, blood was collected from retro-orbital venus plexus of the moderately anaesthetized rats and serum was separated and stored at –20°C for analysis. Radio immuno assay (RIA) of testosterone was carried out using RIA-kit 125_I (Lecco Diagnostic Incorporation, Michigan, USA) (9). Radio activity was determined by the gamma scintillation counter (Electronic Corporation of India, Model 4702). Counting efficiency was 82% of 125_I for single sample.

Gravimetric study : Body weight was recorded twice a week during the experimental period. On 31st day, the animals were autopsied by cervical dislocation and testis cleanly removed and weighed.

Histological study : Five pairs of testis from each group were fixed in Bouin's fluid, dehydrated and embedded in paraffin wax. Sections of 5 µm thick were cut on a microtome (Reichert-Histostat-820) and stained with hematoxylene-eosin (10). The morphometric measurement of Leydig cell nuclear diameter was carried out by using occular micrometer at 640 x magnification and Leydig cell population was analysed per squar cm area at same magnification.

In each case twenty observations were made.

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Different types of nuclear damage in Leydig cells after formaldehyde treatment was calculated in percentage.

Histochemical study : Fresh testicular tissues were quickly transferred in cryostatic microtome chamber (Minotome, IEC, USA) after dissection and fresh frozen section at –20°C of 14 μm thick were placed on glass slides. The sections were incubated in a media containing dehydroepiandrosterone for one hour at 36°C for the localization of 3 β-Δ^5–hydroxysteroid dehydrogenase. The control sections were incubated in a subtrate free media following the same schedule (11).

Few fresh frozen sections on testicular tissue from different groups were immersed in saturated alcoholic Sudan Black-B solution for the localization of lipid materials (12). All histochemical observations were performed at 640 x magnifications.

All data were statistically analysed by Student's 't' test.

RESULTS

Body and organ weight : In groups III and IV highly significant decrease (P<0.001) in body weight was noted as compared with other dose group of formaldehyde (Fig. 1). Similarly, testicular weight was lower at the doses of 10 and 15 mg/kg HCHO (P<0.001) (Fig. 2).

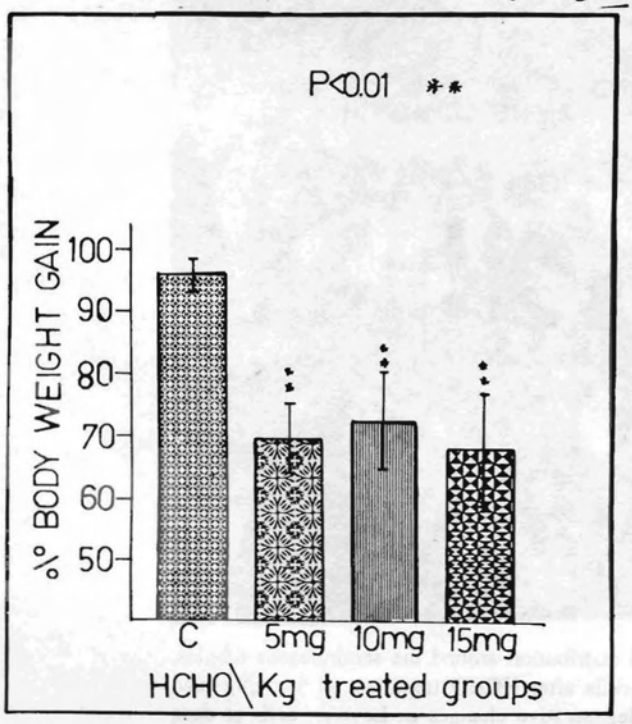

Fig. 1: Effect of formaldeyde on percentage body weight gain of rats. Values are M±SE of ten animals.

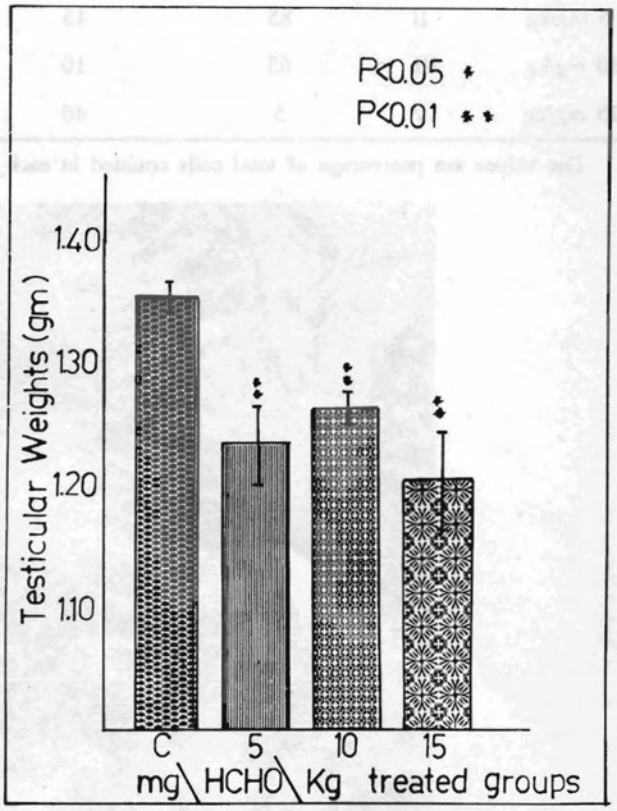

Fig. 2: Formaldehyde induced changes in rat testicular weight. Values are M±SE, n=10.

Histological studies : Testicular tissue of control rats showed normal progressive spermatogenic process alongwith normal Leydig cells (Fig. 3a). The histometric studies in the same group indicated that 90% of Leydig cells were normal among which 10% were Pyknotic (Table I). The Leydig cell population and Leydig cell nuclear diameter were within the normal range (Fig. 4).

Gradual cellular degeneration in seminiferous tubules and also in Leydig cells, given HCHO doses, were demonstrated microscopically (Fig. 3b, c, d). Various types of nuclear damage of Leydig cells was quantitatively determined in the experimental groups.

TABLE I : Various types of nuclear damage in Leydig cells after different doses of formaldehyde treatment in rat.

Doses	*Group*	*Normal (N)*	*Pyknotic (P)*	*Karyochatic (Ki)*	*Karyolytic (Kr)*	*Predominant type*
Control	I	90	10	—	—	N > P
5 mg/kg	II	85	15	—	—	N > P
10 mg/kg	III	65	10	10	15	N > Kr > P, KI
15 mg/kg	IV	5	40	40	15	P, KI > Kr > N

The values are percentage of total cells counted in each group. Leydig cells were counted in 20 field in each case.

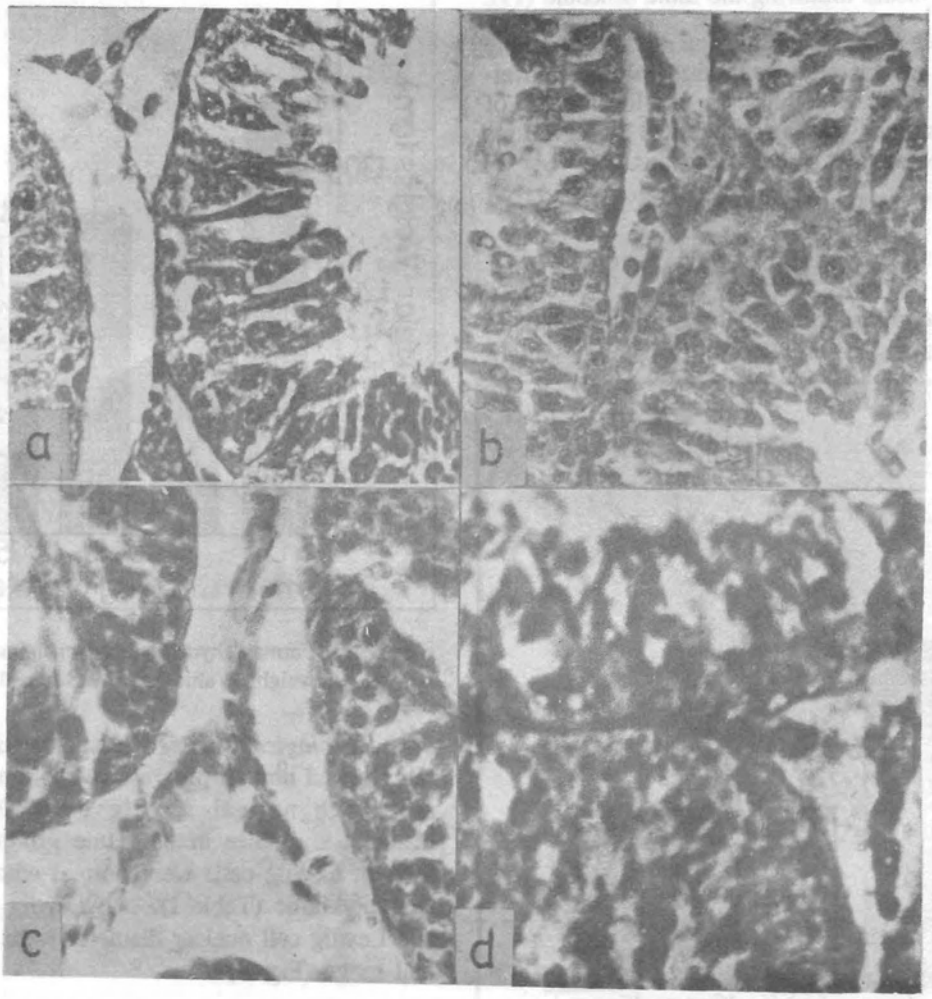

Fig. 3: (a) Showing normal Ledig cell distribution around the seminiferous tubules. (b) Partial damage of Leydig cells after HCHO treatment at 5 mg/kg b.w. for 30 days (c) Progressive degenerative changes in Leydig cells at dose of 10 mg/kg HCHO for 30 days. (d) Complete degeneration of Leydig cells in 15 mg/kg HCHO dose group.

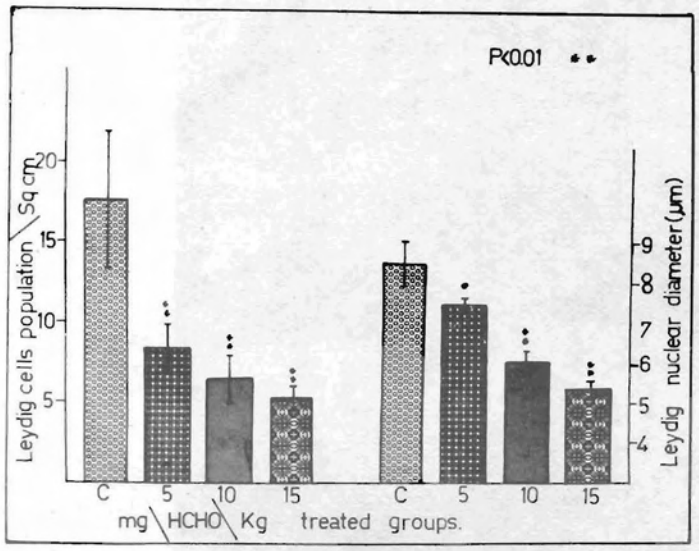

Fig. 4: Leydig cell population and Leydig cell nuclear diameter after different doses of formaldehyde treatment in rats.

Marked nuclear damage in Leydig cells was noted in Group III and IV (Table I). Significant gradual diminution ($P<0.001$) in Leydig cell population and Leydig cell nuclear diameter was observed in all experimental groups (Fig. 4).

Histotochemical studies : In control group, normal reaction intensity of 3β–Δ⁵–hydroxy steroid dehydrogenase (3β–Δ⁵–SDH) was noted in the Leydig cell region. (Fig. 5a)). However gradual decline of histochemical reaction was recorded in groups II, III and IV (Fig. 5b, c, d). Less activity was observed in sudanophillic reaction intensity in testicular tissue in control group, whereas maximum reaction activity was noted in group III and IV which was more than in group II (Fig. 6a, b, c, d).

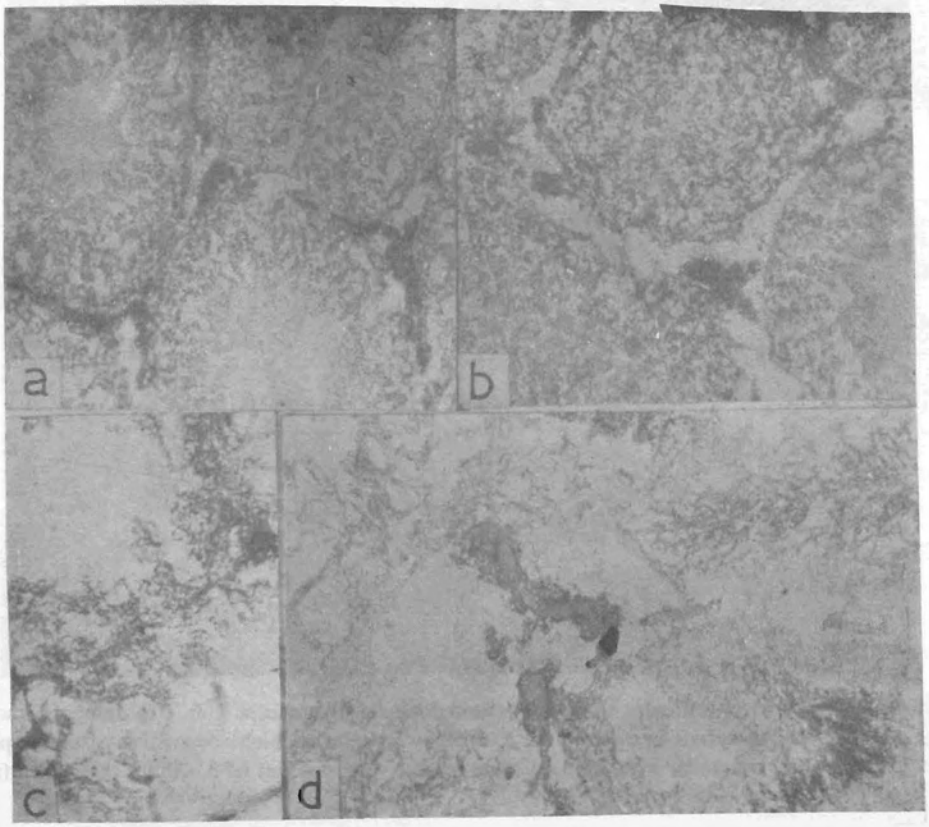

Fig. 5: Effect of formaldehyde on reaction intensity of 3β–Δ⁵-hydroxysteroid dehydrogenenase (3β–Δ⁵-SDH) in rat testicular tissue x 160. (a) Showing normal activity of 3β–Δ⁵-SDH in Leydig cell. (b,c,d) Showing of 5, 10, 15 mg/kg HCHO treatment.

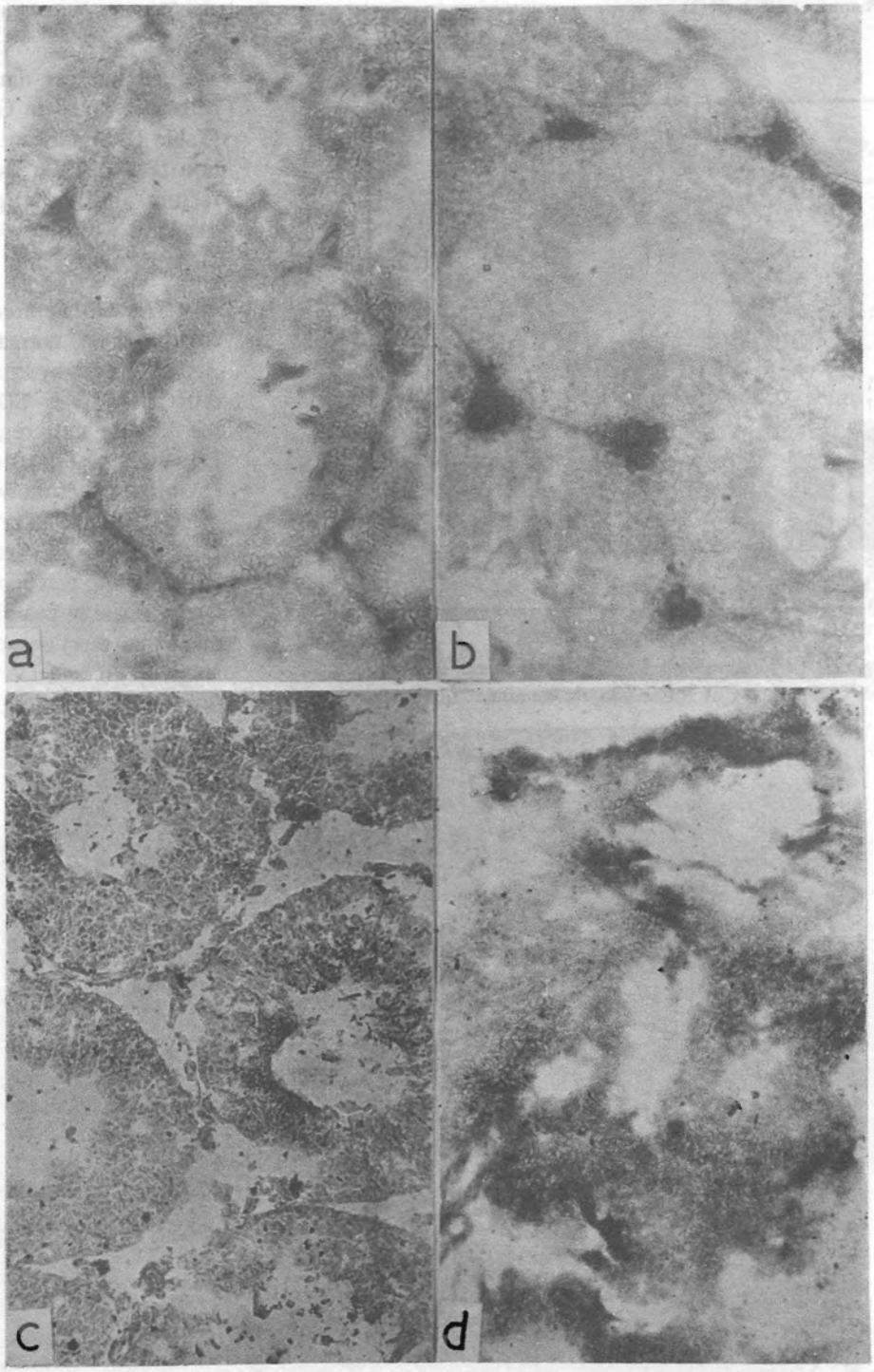

Fig. 6: Sudanophillic reaction in testicular tissue after formaldehyde treatment x 160. (a) Showing low reaction intensity of sudanophillic materials in control. (b, c, d) Showing gradual high deposition of sudanophillic material in testicular tissue after treatment with formaldehyde respectively at doses of 5, 10 and 15 mg/kg (i.p.) for 30 days.

Gradation of histochemical reaction intensity of 3β-Δ⁵-SDH and Sudan was considered from +1 to +6 and recorded in Table II.

TABLE II : Histochemical localization of 3β–Δ⁵-hydroxysteroid dehydrogenase (3β–Δ⁵-SDH) and Sudanophillic reaction (S.R.) intensity.

Doses	*Group*	*3β–Δ⁵-SDH (10)*	*S.R. (10)*
Control	I	+6	+1
5 mg/kg	II	+5	+3
10 mg/kg	III	+3	+5
15 mg/kg	IV	+1	+6

Histochemical reaction intensity were measured +1 to +6. Figures in parenthesis indicate number of observations.

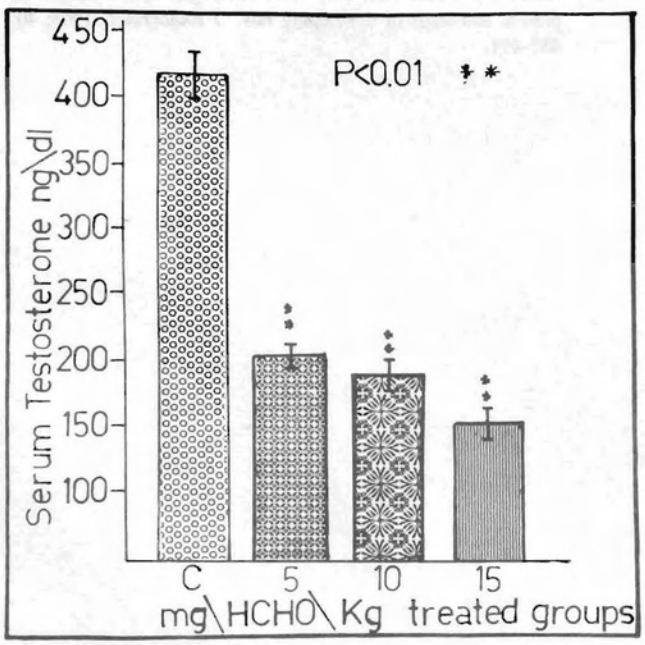

Fig. 7: Effect of formaldehyde on serum testosterone level in different dose group.

Serum testosterone assay : Significant decline of serum testosterone ($P<0.01$) was observed in the experimental groups than in the controls (Fig.7). Groups III and IV showed maximum decline in serum testosterone level.

DISCUSSION

Besides the well known effect of formaldehyde as a potent carcinogen, male steroidogenic impairement was demonstrated in the present study. Gradual decline of body and testicular weight in the experimental groups indicated the cellular growth retardation due to adverse effect of formaldehyde. Cytotoxic effect of formaldehyde has been well documented in literature (13). Arrest of nucleic acid and protein synthesis by HCHO treatment have also been reported earlier (14).

The Leydig cell is a potent androgen producing structure (15). Deformation of Leydig cells after administring different doses of formaldehyde caused diminution of serum testosterone level, which indicates inadequate level of testosterone biosynthesis. Moreover, high lipid accumulation in the Leydig cell region suggests the non-utilization of lipid towards testosterone biosynthesis.

The above observations are in agreement with the gradual inhibition of 3β–Δ⁵–hydroxysteroid dehydrogenase (3β–Δ⁵–SDH) after HCHO treatment. This key enzyme is associated with testosterone hiosynthesis (17). Therefore, the accumulation of lipid materials and decreased activity of 3β–Δ⁵–SDH revealed the inhibition of testosterone biosynthesis alongwith the morphological impairement in the Leydig cells following formaldehyde treatment. Growth retardation may be a causative factor due to androgen deficiency which is an anabolic hormone (18).

In conclusion, formaldehyde treatment in different doses causes different levels of Leydig cell impairment alongwith gradual inhibitory effect on steroidogenesis.

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