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Pathological study on male rats reproductive system effected by trichloroethylene and curcumin

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***Abstract*— The current study evaluated the harmful effects of trichloroethylene and curcumin on the reproductive system in male Wistar rats. For 12 weeks, animals were given 0.2ml/100 g B.W of each (trichloroethylene, curcumin, and corn oil as controls) via gavage. The study found significant differences in testosterone levels among the three groups, with the curcumin group having the highest mean concentration (0.978 ng/ml), indicating a stimulatory or protective effect on testosterone production. Trichloroethylene (TCE) treatment resulted in significantly decreased testosterone levels (0.580 ng/ml), indicating probable endocrine disruption and impaired hormone synthesis. Sperm analysis found that the curcumin group performed better than the TCE and control groups, with higher sperm count, motility, and viability, as well as fewer sperm abnormalities, supporting curcumin's protective effect in sperm quality maintenance. Histopathological tests confirmed these results: TCE exposure produced significant testicular necrosis, congestion, and the degeneration of seminiferous tubules. The curcumin group revealed degenerative alterations but still had some spermatogenic function. The oil control group showed symptoms of testicular degeneration, including macro-vacuolar alterations and a reduction in Leydig cells. Overall, curcumin appears to reduce TCE's reproductive toxicity by increasing hormone levels, sperm parameters, and testicular tissue integrity.**

***Keywords* — Trichloroethylene , Curcumin , male rat, reproductive system. histopathology.**

INTRODUCTION

Environmental factors and insults, such as reproductive toxicant exposure, can disrupt spermatogenesis and cause infertility. Understanding how toxicants interfere with spermatogenesis is crucial for establishing how environmental variables contribute to decreased fertility(1, 2, 3).

Trichloroethane (TCE) is frequently used as an industrial solvent and degreaser (4). TCE is requested to be well absorbed through all exposure routes. Several studies with developmental exposure to lower concentrations than the "safe" dose suggest that TCE exposure can produce a variety of negative abnormalities birth defects, including low fetal weight, and developmental issues. It has recently been demonstrated that chemical exposure on a daily basis is connected with an increased risk of infertility, birth abnormalities, and low fetal weights (5).

Exposure to TCE has been shown to interfere with the endocrine system, potentially disrupting hormone levels such as testosterone. This can lead to alterations in reproductive functions and behaviors (6).

Studies have reported that exposure to TCE can negatively impact sperm quality, leading to decreases in sperm count, motility, and viability. These changes can adversely affect fertility (7).Repeated exposure to TCE may result in pathological changes in the testes, including alterations in seminiferous tubule structure, reduced testicular weight, and impaired spermatogenesis, the process by which sperm is produced (8).

The mechanisms by which TCE exerts its toxic effects are not fully understood but may involve oxidative stress, genotoxic effects, and direct actions on reproductive tissues (9). the genotoxic effects of TCE have direct implications for reproductive health. DNA damage in germ cells can interfere with spermatogenesis, affecting sperm quality and function. Such effects can lead to decreased fertility and increased risk of developmental abnormalities in offspring (10).

Curcumin (Cur) is a polyphenolic compound extracted from the edible spice turmeric with various pharmacological effects (11, 12). It plays an important role in Indian and Chinese traditional medicine for thousands of years (13, 14).

In addition, experiments have shown that curcumin is safe in humans even at high doses (12 g/day) (11). In recent years, curcumin has received more and more attention from scientists due to its anti-inflammatory, antioxidant, and antitumor effects(15). Studies have demonstrated promising therapeutic

outcomes in cancer, cardiovascular disease, and immune disease including arthritis, uveitis, ulcerative proctitis, and tropical pancreatitis (16). CUR has been considered to have anti-inflammatory and antioxidant properties (17, 18).

The capability of cur to prevent tumor formation, forestomach, duodenum, and colon, as well as rats' tongue, colon, mammary glands, and sebaceous glands, is well proven (19). CUR also has been shown to minimize lipid peroxidation produced by numerous toxic substances in hepatocytes, both in vitro and in vivo (20, 21).Curcumin induces the expression of antioxidant enzymes, reducing oxidative stress in reproductive tissues. It helps scavenge free radicals and enhances the activity of endogenous antioxidants like superoxide dismutase (SOD) and glutathione peroxidase (GPx), which in turn protects against oxidative damage to sperm cells and testes (22).

Curcumin inhibits pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). By downregulating inflammation in testicular tissues, it helps maintain a favorable environment for spermatogenesis and protects against inflammation-induced damage (23). Curcumin has been shown to influence the levels of key reproductive hormones, including testosterone. It may enhance testosterone production by stimulating Leydig cells, thereby promoting spermatogenesis and improving overall male reproductive function (24). Curcumin can protect against DNA damage in spermatozoa caused by environmental toxins and oxidative stress. By enhancing the DNA repair mechanisms in germ cells, curcumin reduces the risk of mutations and improves sperm quality (25). So the aim of this study is to determine the reproductive toxicity of TCE in male rats and to evaluate curcumin's preventive properties in reproductive system.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for the study was obtained from the local Animal Care and Use Committee, College of Veterinary Medicine, University of Baghdad. under approval number 1388/P.G., dated 29-7-2024.

A total of 30 healthy adult male albino rats, aged about three months and weighing 250- 300 g, were utilized in this experiment. The rats were bred and maintained at the animal house, College of Veterinary Medicine University of Baghdad. Rats were acclimated for 4 weeks in traditional cages (20×30×50 cm) with 5 rats per cage under control environmental conditions of 22 (±3) °C, 50 (±5)% of relative humidity, and a 12/12-h light/dark cycle. Normal feed pellets and water were provided ad libitum during the experiment (26).The rats were randomly divided into three groups of ten each of them. The control group administered corn oil orally for 12 weeks to establish baseline responses. For 12 weeks, the TCE-treated group received a sub-lethal dose of 1/10 of the LD50 (0.2 mg/kg BW) in the form of 0.2 mL to 100 gm/BW curcumin mixed with corn oil also for 12 weeks.

Blood samples collected and placed in dry sterile tubes then centrifuge to obtain the serum after that hormones were measured (27, 28). However serum level of testosterone hormones was assessed according to (29).

After the experiment, the rats were euthanized, and sperm was extracted from the epididymal tail. The cauda epididymis was dissected and immersed in 1 mL of pre-warmed normal saline (N.S). A Neubauer chamber was used to estimate the complete sperm count in one drop of sperm suspension. Smooth agitation and tissue tearing were used to cause the spermatozoa to swim out into the N.S., and the sperm mixture was deposited in an Eppendorf tube in a rat. Samples were incubated at 37°C with 5% CO2 for 20 minutes for sperm analysis (30, 31).

Semen collection and analyses:

In order to get the sperm to become motile and swim out from the right epididymis, Rats from each of the three groups had their right epididymis removed and cut into many pieces on a test bottle filled with normal saline for (1-2) minutes. After that, a clean slide was placed on which the semen was deposited and covered with a cover slip using a 1 ml pipette. According to Beltrame and Sasso(32), the slides were examined for sperm quantity and motility using a light microscope. under power (40X).

The Sperm total count procedure described by (33, 34) was used to determine the total quantity of sperm. It involved removing the epididymis and cutting it into tiny pieces, which were then placed in a specific container with 1ml of phosphate regulator solution (pH 7.2) to create a suspension solution. After filtering the suspension using a specialized strainer, the sperm are counted using a hemocytometer (Neubauer's chamber). Leachate liquid was taken out using a white cell pipette to the 0.5 mark, and the phosphate buffer solution was then used to finish the size to 11. According to the following equation, the amount of sperm in 8 square meters was converted to sperm per milliliter: Total count of sperm = number of sperm × 5×10^4

Sperm abnormalities a drop of the suspension was mixed with a drop of hematoxylin-eosin stain,and a light swab was applied and allowed to dry for the examination of abnormal sperm morphology in cases where the semen sample was obtained in the same manner as the total number of sperm (35).

Sperm viability was evaluated using the traditional gold standard procedure that involved the detection of eosin, which penetrates the membranes of injured cells. Approximately 100 µl of sperm suspension was combined with two drops of 1% eosin and left for 30 seconds. The solution was then mixed with three drops of 10% nigrosin, and a thick smear was made in triplicate within 30 seconds. The results were obtained by detecting both motile and immotile sperm and are shown as a percentage (%)(36).

For histopathological examination After 12 weeks of treatment, rats were anesthetized and the testes were preserved in 10% formalin for histological analysis and the standard hematoxylin and eosin staining procedure. The specimens were cleaned overnight with running tap water to remove the formalin. They were dehydrated in an escalating series of alcohols, processed through xylene-alcohol, and cleared in two 30-minute xylem changes. They were immersed in a mixture of xylene and melted paraffin for an hour before being immersed in two pure paraffin changes for 30 minutes each for

infiltration. The specimens were encased in pure paraffin to produce blocks. Serial sections were cut with a rotary microtome to a thickness of 5 microns. Sections were stained in haematoxylin and eosin according to (37, 38). Statistical analysis

The Statistical Packages of (39) program was used to detect the effect of difference groups in study parameters. Least significant difference-LSD test was used to significant compare between means in this study.

RESULT & DISCUSSION

Testosterone levels differ significantly among the three groups, according to the findings as a show in (table 1) Curcumin had the greatest mean testosterone concentration (0.978 ng/ml), which differs considerably from both Trichloroethylene (0.580 ng/ml) and the Control group. This shows that curcumin may stimulate testosterone synthesis or have a protective impact when compared to other groups, while Trichloroethylene had showed the lowest average testosterone concentration, considerably lower than both curcumin and control. This implies that trichloroethylene may impede testosterone synthesis or metabolism. Furthermore The control group has an intermediate testosterone levels, which is considerably greater than trichloroethylene but not statistically different from curcumin.

Table 1. Comparison of different groups in Testosterone hormone concentration.

Group	Mean ±SE of Testosterone (ng/ml)
G1	0.758 ±0.08 ab
G2	0.580 ±0.06 b
G3	0.978 ±0.12 a
L.S.D.	0.273 *
P-value	0.0241
This means that having different letters in the same column differed significantly * (P≤0.05). G1: Control , G2: Trichloroethylene , G3: Curcumin	

Semen Analysis

The (G2) had significantly lower sperm counts than the (3) and (G1) (P≤0.01). G3 had the highest count, indicating that it stimulates sperm production. While G3 had a significantly decreased percentage of aberrant sperm compared to G2 (P<0.01). This suggests that curcumin may protect against sperm abnormalities. Furthermore G3 significantly lowers the percentage of dead sperm compared to G2 and G1 (P≤0.01), suggesting a protective effect for sperm survival. G3 significantly increased sperm motility compared to G2 and G1 (P≤0.01), indicating that it improves sperm function (Table-2).

Table 2. Comparison between difference groups in Sperm parameters

Group	Means ±SE			
	Sperm count ($x10^4$)	Abnormality (%)	Dead (%)	Motility count (%)
(G1) Control	175.16 ±16.02 a	36.33 ±8.02 b	63.67 ±4.51 a	35.00 ±4.35 b
(G2) Trichloroethylene	79.50 ±0.06 b	55.67 ±2.79 a	62.16 ±4.84 a	38.83 ±4.71 b
(G3) Curcumin	202.00 ±14.11 a	18.83 ±2.27 c	36.67 ±2.97 b	63.50 ±2.67 a
L.S.D.	38.43 **	15.30 **	12.62 **	12.08 **
P-value	0.0001	0.0005	0.0005	0.0003
Means having with the different letters in same column differed significantly. ** (P≤0.01).				

Pathological study:

Grossly results shows congested and edematous testes of G1 (corn oil)Fig 7. ,while results of G2(TCE) shows severe congested blood vessels of the testes(TCE group) (Fig 8.). Histopathological examination Figure 9. Corn oil group shows epididymis tubules with macro-vacuolar degeneration red arrow)and debris inside lumen (blue arrow) .G2 (TCE) as shown in blue rows there is severe distraction of seminiferous tubules and necrosis of the germ cells (blue arrow) Fig10., Figure 11. Histopathological section of testes G2 shows seminiferous tubules with immature rounded sperms and debris (black arrow), congested blood vessels(green arrow). Histopathological section of testes G3 (cur) shows seminiferous tubules with degenerative changes and debris in the lumen (red arrow) and necrosis of ladyig cells (blue arrow) Fig 12. , in other hand testes of G3 (cur) shows seminiferous tubules with severe degeneration changes and no spermatogenesis(blue arrow) Fig 13.

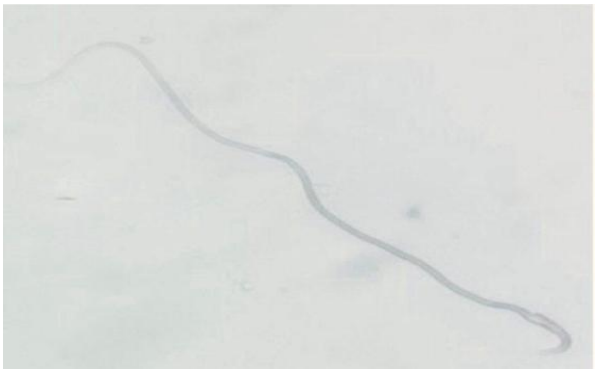

Figure 1: normal sperm

Figure.2 abnormal sperm (curved tial)

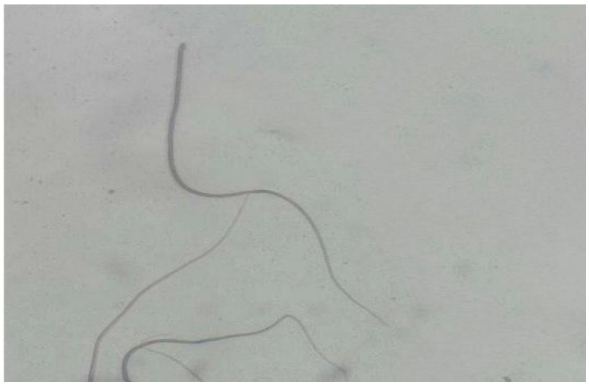

Figure 3. Abnormal sperm (absent head)

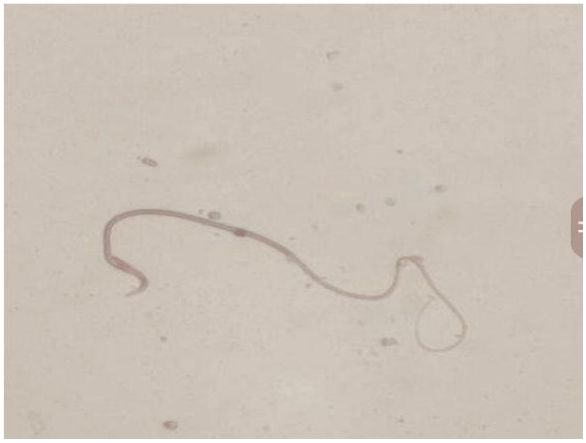

Figure 4. abnormal sperm colloid tail

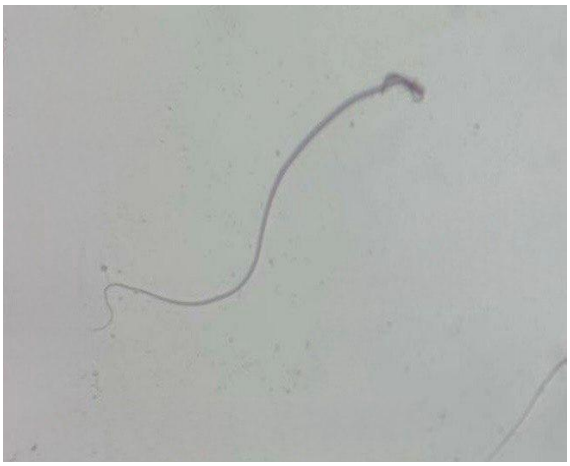

Figure 5. amorphous head

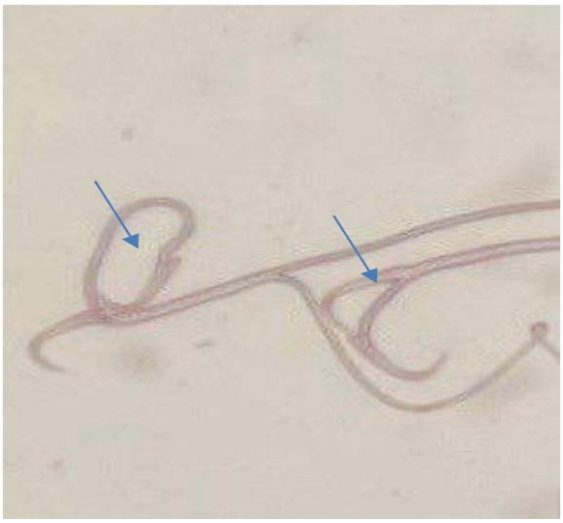

Figure 6. colloid neak and double head

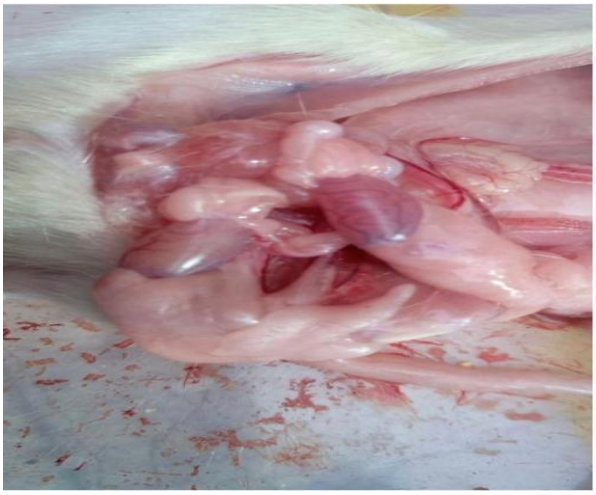

Figure 7. congested and odematous testes of group(control)

Figure 8. congested blood vessels of the testes(TCE group)

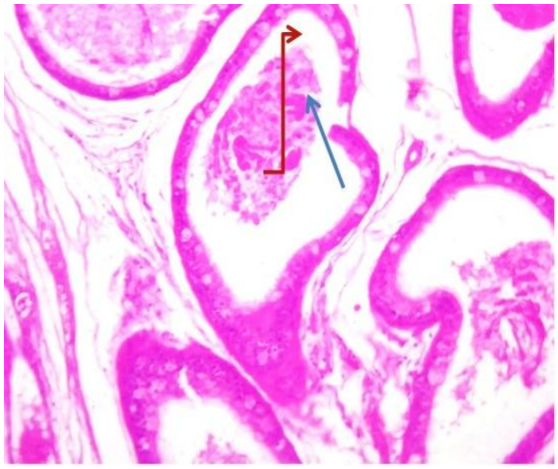

Figure 9. Corn oil group shows epididymis tubules with macro-vacuolar degeneration red arrow)and debris inside lumen (blue arrow) (H&Estain 100X)

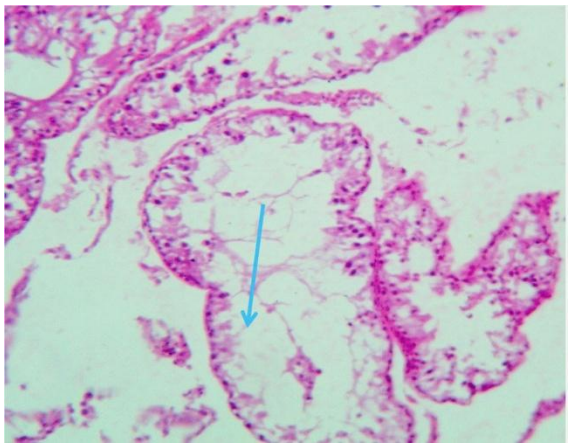

Figure 10. Histopathological section of testes (TCE) group as shown in blue rows there is severe destraction of seminiferous tubules and necrosis of the germ cells (blue arrow) (H&Estain 100X)

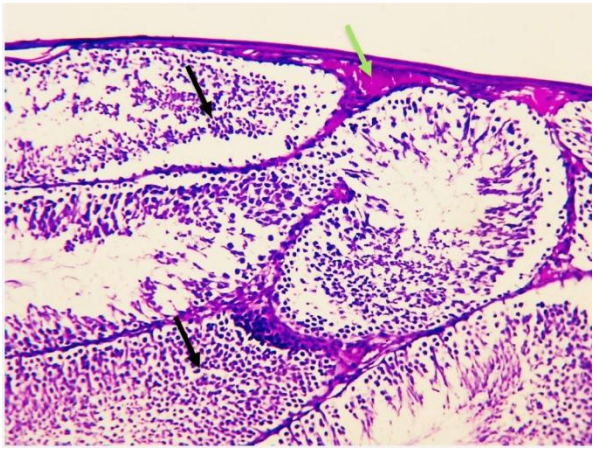

Figure 11. Histopathological section of testes (TCE) group shows seminiferous tubules with immature rounded sperms and debris (black arrow),congested blood vessels(green arrow). (H&Estain 100X)

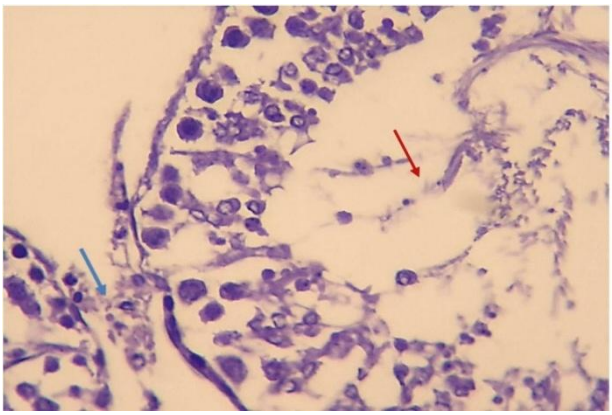

Figure 12. Histopathological section of testes (cur) group shows seminiferous tubules with degenerative changes and debris in the lumen (red arrow) and necrosis of ladyig cells(blue arrow). (H&Estain 400x)

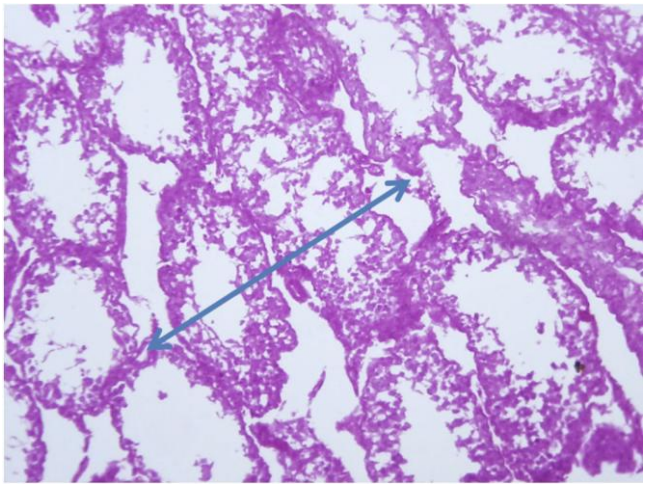

Figure 13. Histopathological section of testes (cur) group shows seminiferous tubules with severe degeneration changes and no spermatogenesis(blue arrow).
(H&Estain 100X)

The data imply that the control group's intermediate values show that baseline testosterone is unaffected by external interventions but remains susceptible to environmental contaminants High amounts of corn oil may have negative effects, such as increased oxidative stress, which can impair sperm motility and morphology; it may also change hormone levels, affecting testosterone synthesis and, as a result, spermatogenesis (40). In contrast, the significantly lower testosterone levels in the Trichloroethylene (TCE) group (0.580 ng/ml) may reflect TCE's known toxicity and endocrine-disrupting potential, which impairs testosterone synthesis by damaging Leydig cells or interfering with steroidogenic enzymes (41, 42).while the curcumin group may have a favorable effect on testosterone levels, possibly due to its antioxidant and anti-inflammatory qualities, which are known to enhance endocrine function (16,43). The curcumin group had the highest mean testosterone concentration (0.978 ng/ml), which is consistent with studies demonstrating that curcumin improves gonadal function and steroidogenesis (44).Turmeric has been reported to reduce testosterone levels and sperm movement in men (45).

Many studies demonstrated that rats exposed to corn oil expiated significant reduction in both sperm motility and concentration along with an increase in oxidative stress markers (46, 47). The significant decline in sperm count and increased abnormalities in the Trichloroethylene (TCE) group align with existing evidence that TCE acts as an endocrine disruptor and toxicant, impairing spermatogenesis and causing oxidative damage to sperm cells (48,49). The data indicate that curcumin has a notable positive effect on various parameters of semen quality. Its ability to increase sperm count, reduce sperm abnormalities, decrease sperm mortality, and enhance motility suggests that curcumin supports spermatogenesis and sperm function, likely due to its antioxidant and anti-inflammatory effects (50, 51). The protective effects observed with curcumin may stem from its capacity to neutralize reactive oxygen species, thereby protecting testicular tissue and spermatozoa from oxidative stress, which is a major factor in sperm damage (52).

It has been demonstrated that both dietary components and environmental contaminants have a major impact on reproductive health. Corn oil, for example, can have both positive and negative effects in rats depending on the dosage and total dietary balance (53,54). The results show significant variations in sperm parameters between treatment groups. Trichloroethylene: This group's significantly lower sperm count, higher abnormality rates, higher mortality rates, and reduced motility indicate that Trichloroethylene has a negative impact on sperm health. Previous research has found that exposure to various environmental toxins, including Trichloroethylene, can have a negative impact on reproductive health and sperm quality due to oxidative stress and cellular damage (55). Sperm count and morphological changes: In rats, exposure to trichloroethylene (TCE) has been linked to lower sperm counts and increased aberrant sperm morphology, indicating negative impacts on male reproductive health. (56,57).

Curcumin has strong antioxidant qualities, which may help protect sperm cells from oxidative damage induced by environmental pollutants or nutritional imbalances (58). Anti-Inflammatory Effects: Curcumin has the potential to lessen reproductive tract inflammation, which would enhance sperm quality by favorably affecting spermatogenesis (59). a Frequency of studies have tried addressing the pharmacokinetics of that is poorly absorbed from intestine after oral administration of different doses of curcumin in rats (60). It was shown that oral consumption of curcumin in rats resulted in approximately 75% being excreted in the feces and only traces appeared in the urine (61).

The observed testicular and epididymal histopathologies reflect toxic effects typical of EDCs (Endocrine Disrupting Chemicals) like TCE induces oxidative stress, leading to germ cell apoptosis and Sertoli/Leydig cell damage (62). Vacuolar degeneration in the epididymis implies a disruption in sperm maturation, which is essential for fertility. Toxic chemicals

such as trichloroethylene (TCE) can cause spermatotoxicity in rats by causing oxidative damage to germ cells and disrupting hormonal balance, resulting in low sperm quality (63, 64).

CONCLUSION

The severity of corn oil exposure varies according to factors such as oil content, individual characteristics, and environmental conditions. TCE has a deleterious influence on male reproductive health by producing oxidative stress, tissue damage, and hormone disturbance, whereas curcumin provides protective advantages by increasing testosterone, sperm quality, and testicular function via its antioxidant and anti-inflammatory characteristics.

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