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Protective and Ameliorative Effects of *Tribulus terrestris* on Cyproterone Acetate-Induced Reproductive and Endocrine Toxicity in Albino Rats

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ABSTRACT

Cyproterone acetate (CPA) is an artificially produced anti-androgen that is frequently used clinically to treat conditions associated with androgen. Nevertheless, prolonged administration of CPA produces significant toxicity to the reproductive and endocrine systems. The aim of the current study was to evaluate the protective and ameliorative potential of *Tribulus terrestris* (a widely known medicinal plant) against CPA-induced reproductive dysfunction in male albino rats. The male albino rats used were *Rattus norvegicus* and were divided into four groups (Control Group, CPA-treated Group, *Tribulus terrestris* Group, and Combined Treatment Group). Following an oral dose of CPA (10 mg/kg body weight) and *Tribulus terrestris* extract (100 mg/kg body weight) for thirty days, various parameters were examined; body weight, testes weight, sperm count and motility, serum testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH), oxidative stress markers (Malondialdehyde (MDA) levels and Superoxide Dismutase (SOD) activity). After performing a histopathological analysis of the testicular tissue all test subjects were examined and it was found that CPA had a very significant detrimental effect on reproductive hormones and parameters and increased the amount of oxidative stress within the body. However, if we took *Tribulus terrestris* simultaneously with CPA, we could restore sperm parameters, hormones, and antioxidant levels back to pre-treatment level (within normal limits). The histological analysis showed that seminiferous tubules (where sperm are produced) were beginning to recover in the treatment groups that received both *Tribulus terrestris* and CPA. Thus, the results from this study indicate that *Tribulus terrestris* can protect against CPA toxicity to reproduction and endocrine systems, and provide significant evidence to support using herbal remedies as treatment options to reduce pharmaceutical-induced reproductive damage. In addition, there is substantial evidence from this study to support that *Tribulus terrestris* could be helpful in preventing further damage by serving as an all-natural protective agent.

Introduction

The sustainability of reproductive health is one aspect of overall physical health, but it can be affected by the environment, chemicals, and pharmaceutical medications. Many synthetic (man-made) medications are effective for their intended purposes, but may have negative effects on reproduction and/or the endocrine system if the medications are taken for a long time, or at high dosages. Cyproterone acetate (CPA) is a synthetic anti-androgen, a type of medication used to treat prostate cancer and hirsutism (excessive hair), as well as acne and other conditions related to male hormones (Nieschlag & Behre, 2010). CPA works by blocking androgens (male hormones) from working at androgen receptors and also decreases adequate levels of gonadotropin which derails testosterone production. Although both of these actions provide a therapeutic benefit, they can also change normal reproductive physiology by decreasing the amount of sperm produced, by decreasing the ability to produce sperm, and by creating an imbalance of hormones (Kumar *et al*., 2015). Prolonging exposure to CPA decreases testicular size, the seminiferous tubules (the tubes in which sperm is formed) atrophy, and reduces the ability to reproduce (Sharpe, 2018). In the past few years there has been an increased interest in using herbal medicine as a possible alternative to pharmaceuticals in this regard. *Tribulus terrestris* is a plant that has a long history of being used in both Ayurveda and Traditional Chinese Medicine and has a noted ability to work as an aphrodisiac, antioxidant, and adaptogen (Adaikan *et al*., 2000). Bioactive materials such as flavonoids, saponins (mainly protodioscin), alkaloids and glycosides present in the plant are responsible for their pharmacology-related activity. *Tribulus terrestris* has been demonstrated in numerous studies to assist with enhancement of testosterone levels, improve sperm quality, and provide an antioxidant effect by protecting against oxidative stress (Neychev & Mitev, 2005). The mechanism of oxidative stress is one of the primary causes that leads to reproductive toxicities caused by drugs and is characterised by excess reactive oxygen species formed that lead to lipid peroxidation, as well as protein and genetic material damage (Aitken & Roman, 2008). The number of people who suffer from drug-induced reproductive disorders continues to rise and therefore the need for safer therapeutic options is essential. The study presented herein will examine the beneficial and protective effects of the plant, *Tribulus terrestris* against CPA-induced reproductive and endocrine toxicity in albino rats.

Review of Literature

Anti-androgens harm male reproduction. Cyproterone acetate lowers testicular weight, sperm counts and testosterone (Toppari 2010). Cyproterone deprives the hypothalamic-pituitary-gonadal (HPG) axis and inhibits Leydig cell function. Klinefelter (2012) found that anti-androgens degenerate seminiferous tubules and inhibit spermatogenic cells. Histological studies indicate that CPA-

treated animals have vacuoles, reduced germ cells and poor spermatogenesis. Possible improvement in reproductive health occurs with medicinal plants, particularly *Tribulus terrestris* . Adaikan *et al*. (2000) showed that T. terrestris increased androgen receptor density and testosterone levels. Neychev and Mitev (2005) report inconsistent results based on the amount and time of T. terrestris treatment. Several studies have confirmed that Tribulus could provide anti-oxidative effects. The potential antioxidant activity of the extract has been documented through studies indicating its ability to stimulate antioxidant enzymes like SOD, catalase and glutathione peroxidase that cause oxidative damage (Zhu *et al*. 2017). More recently, interest has developed surrounding the use of herbal products as protective agents against drug induced toxicity, where Singh *et al*. (2020) indicated that there is significant reduction in oxidative stress and reestablishment of normal histological architecture within reproductive tissues when using plant derived antioxidants. However, only limited research exists evaluating the combined effects of *Tribulus terrestris* and CPA. Thus, the current study attempts to address this void by assessing both the protective and ameliorative properties of the extract.

Materials and Methods

Experimental Animals- Thirty-two adult male albino rats (*Rattus norvegicus*), weighing 150–200 g, were used. Animals were housed under standard laboratory conditions with a 12-hour light/dark cycle and free access to food and water.

Experimental Design- Animals were divided into four groups (n=8):

Group I (Control): Normal saline

Group II (CPA): 10 mg/kg CPA

Group III (TT): 100 mg/kg *Tribulus terrestris*

Group IV (CPA + TT): Combined treatment

Drug and Extract Administration

CPA and plant extract were administered orally for 30 days.

Parameters Studied

Body weight and organ weight, Sperm count and motility, Hormonal assays (Testosterone, LH, FSH), Oxidative stress markers (MDA, SOD)

Histopathology- Testicular tissues were fixed in formalin, processed, sectioned, and stained with hematoxylin and eosin.

Statistical Analysis- Data were expressed as Mean ± SD. One-way ANOVA followed by Tukey's test ($p < 0.05$) was used.

Results

Table 1: Reproductive Parameters

Group	Sperm Count (million/ml)	Motility (%)	Testosterone (ng/dL)
Control	65 ± 4	80 ± 3	5.8 ± 0.3
CPA	30 ± 3*	45 ± 4*	2.1 ± 0.2*
TT	68 ± 5	82 ± 3	6.0 ± 0.4
CPA+TT	55 ± 4#	70 ± 3#	4.5 ± 0.3#

(*Significant vs control, #Recovery vs CPA)

Table 2: Oxidative Stress Markers

Group	MDA (nmol/mg)	SOD (U/mg)
Control	2.5 ± 0.2	8.0 ± 0.4
CPA	5.8 ± 0.3*	4.2 ± 0.3*
TT	2.3 ± 0.2	8.5 ± 0.5
CPA+TT	3.0 ± 0.2#	7.2 ± 0.4#

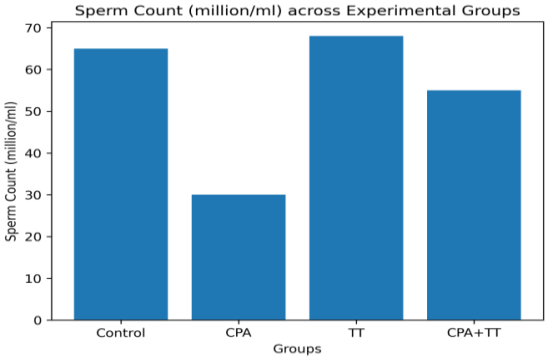


Figure 1. Effect of *Tribulus terrestris* on sperm count in cyproterone acetate-treated albino rats. Values are expressed as Mean ± SD (n=8). *Significant decrease compared to control ($p < 0.05$); #Significant recovery compared to CPA group.

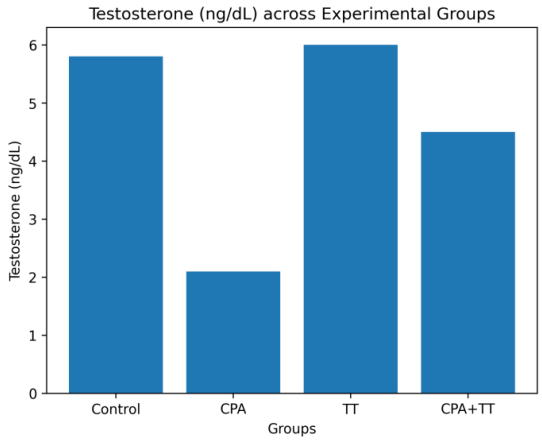


Figure 2. Serum testosterone levels in different experimental groups. *Tribulus terrestris* significantly restored testosterone levels reduced by cyproterone acetate. (*$p < 0.05$ vs control; #$p < 0.05$ vs CPA).

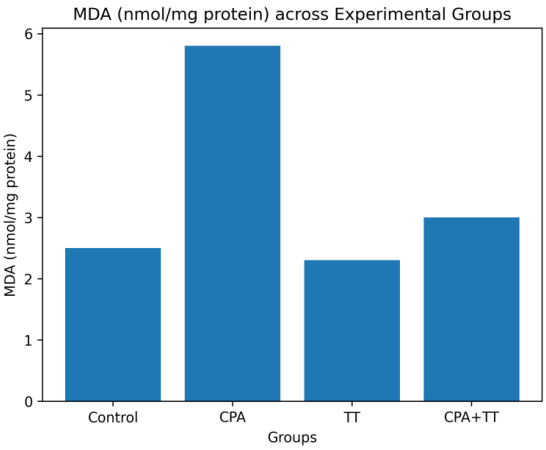


Figure 3. Lipid peroxidation levels (MDA) indicating oxidative stress. CPA significantly increased MDA levels, while *Tribulus terrestris* reduced oxidative damage.

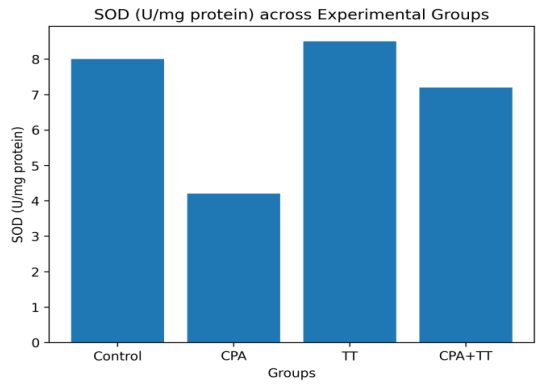
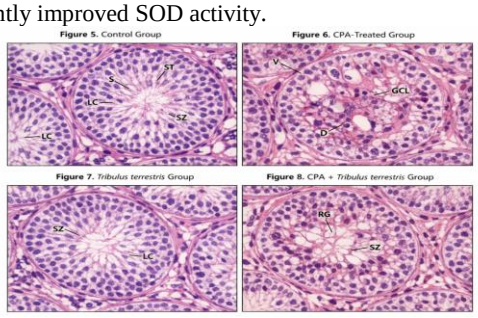


Figure 4. Superoxide dismutase (SOD) activity in experimental rats. CPA reduced antioxidant defense, while *Tribulus terrestris* significantly improved SOD activity.



Histopathological Observations

ST → Sperm formation site, LC → Testosterone production

SZ → Mature sperm, V + D → Damage indicators

RG → Recovery indicator

Control Group-Testicular sections of control rats showed normal architecture with well-organized seminiferous tubules. Germinal epithelium was intact with all stages of spermatogenic cells, including spermatogonia, spermatocytes, spermatids, and spermatozoa. Leydig cells were clearly visible in the interstitial spaces, indicating normal testosterone production.

CPA-Treated Group- Marked histopathological alterations were observed:

- Disorganization of seminiferous tubules
- Reduction in germ cell layers
- Vacuolization in Sertoli cells
- Degeneration of spermatogenic cells
- Reduced lumen sperm density

These changes indicate severe impairment of spermatogenesis and confirm reproductive toxicity.

- *Tribulus terrestris* Group
- Testicular sections showed:
- Normal seminiferous tubules
- Active spermatogenesis
- Dense spermatozoa in lumen

No pathological alterations were observed, indicating safety of the plant extract.

CPA + *Tribulus terrestris* Group

Significant recovery observed:

- Restoration of seminiferous tubule structure
- Increased germ cell population
- Reduced vacuolization
- Improved sperm density

Figure 5. Photomicrograph of control rat testis showing normal seminiferous tubules with complete spermatogenic series (H&E, 400×).

Figure 6. CPA-treated group showing degeneration of seminiferous tubules, reduced germinal epithelium, and vacuolization (H&E, 400×).

Figure 7. *Tribulus terrestris* -treated group showing normal testicular architecture and active spermatogenesis (H&E, 400×).

Figure 8. CPA + *Tribulus terrestris* group showing recovery of seminiferous tubules and restoration of spermatogenic cells (H&E, 400×).

Discussion

This research has shown that cyproterone acetate (CPA) significantly affects the reproductive and endocrine systems of albino rats based on the changes in the sperm parameters, hormone levels, oxidative stress, and the histopathological changes in testicular tissue. These studies align with previously published literature showing that anti-androgen medications impact male reproductive physiology via various mechanisms (e.g., inhibiting the hypothalamic-pituitary-gonadal (HPG) axis and affecting testicular functions directly) (Toppari *et al*., 2010; Sharpe, 2018). The decrease in the number of spermatozoa and motility due to the administration of CPA may be attributed to the effects of CPA on the regulation of spermatogenesis and Sertoli cell function. Spermatogenesis relies on adequate testosterone levels and the normal functioning of the seminiferous tubules. By effectively acting as anti-androgens, CPA competes with endogenous androgens at receptor sites and inhibits gonadotropin release from the pituitary, resulting in decreased testosterone production (Nieschlag & Behre, 2010). Ultimately, the low testosterone levels help to limit germ cell replication and differentiation and, therefore, sperm volume produced (Kumar *et al*., 2015). Furthermore, the noticeable reduction in serum testosterone levels seen in CPA-treated animals demonstrates that the drug was responsible for disrupting endocrine function. Testosterone is one of the hormones responsible for regulating male reproductive function (i.e., spermatogenesis); libido and the development of secondary sexual traits are also dependant upon sufficient levels of testosterone. CPA also has been shown to inhibit the secretion of luteinizing hormone (LH) from the pituitary gland and subsequently reduce Leydig cell drive and production of testosterone (Toppari *et al*., 2010). Additionally, the reduction of follicle-stimulating hormone (FSH) reduces the ability of Sertoli cells to support the needs of spermatogenic cells. A similar disruption of hormones has been documented in studies on anti-androgenics, where suppression of the HPG axis led to infertility and testicular atrophy (Sharpe, 2018). Oxidative stress is also an essential contributor to CPA-induced toxicity. The current study documented that CPA-treated rats had statistically significant increases in malondialdehyde (MDA) levels and decreases in superoxide dismutase (SOD) activity. MDA is a well-established indicator of lipid peroxidation; it serves as a marker of injury to cellular membranes from free radicals, while SOD serves as an important antioxidant enzyme responsible for neutralizing reactive oxygen species (ROS). Oxidative stress occurs when the quantity of ROS produced exceeds the antioxidant defenses, leading to oxidative damage to sperm DNA, proteins and lipids, subsequently reducing sperm function (Aitken & Roman, 2008). The susceptibility of the testis to oxidative damage is due to the high rate of cell division in the testis and high concentrations of polyunsaturated fatty acids in the sperm cell membrane. Increased amounts of ROS are also associated with apoptosis of germ cells and degeneration of seminiferous tubules (Klinefelter *et al*., 2012). Evidence of these effects has been observed in the present study in rats treated with CPA (cytochrome P450 antagonist). The CPA-induced disorganisation of seminiferous tubules, decreased number of germ cell layers and vacuolisation shown by histopathological results, supports this mechanism of oxidative damage in rats treated with CPA. Additionally, *Tribulus terrestris* administration with CPA showed improvements in reproductive parameters, hormone levels and antioxidant status. These results indicate that *Tribulus terrestris* has protective and ameliorative effects against CPA-induced damage on reproduction. The increased sperm count and motility observed in the combined treatment group indicate a restoration of spermatogenic activity and may be due to steroidal saponins, in particular, protodioscin, which have been reported to stimulate the production of androgens and enhance reproductive function (Adaikan *et al*., 2000). The rise in testosterone levels seen in the group treated with *Tribulus terrestris* confirms once again that it has the potential to naturally enhance androgens. A multitude of studies support this claim by showing that *Tribulus terrestris* induces releases of luteinizing hormone, and subsequently boosts testosterone synthesis from Leydig cells (Neychev & Mitev, 2005). While some studies have had varied findings regarding its androgenic properties, the current investigation produced clear evidence as to its efficacy in providing hormonal equilibrium after being estrogenically suppressed by drugs. In conjunction with its hormone-elevating properties, *Tribulus terrestris* exhibited strong antioxidant properties. The reduction in MDA and improvement in superoxide dismutase activity from the combination of treatment indicates that the Tribulus extract is effective in decreasing oxidative stress. Several flavonoids and phenolic compounds contained within the plant extract are responsible for the antioxidant property, as they can scavenge free radicals and improve endogenous antioxidant defenses (Zhu *et al*., 2017). By lowering the level of oxidative stress, *Tribulus terrestris* protects the integrity of cellular structures and prevents cellular damage from occurring to both sperm cells and tissues associated with the testes. The histopathological findings from this investigation also supported the protective nature of *Tribulus terrestris* . Both CPA and the plant extract improved the testical structure and restoration of the seminiferous structure and number of spermicogenic cells, showing that the plant extract is not only able to prevent damage, but also may stimulate regrowth of reproductive structures. Studies have also found similar results when utilizing herbal antioxidants as counteracting agents for drug-induced testicular toxicity with their use (Singh *et al*., 2020). Another critical feature of this study is that *Tribulus terrestris* has dual protective and ameliorative effects. That is, the protective effect is present in that it prevented or decreased the amount of damage produced by CPA when

administered with CPA, and the ameliorative effect is present in that it restored normal physiologic function after damage occurred. The two-fold action of the Tribulus terestris plant gives it a broader therapeutic use as an effective agent for managing drug-induced reproductive disorders. The findings in this study further support the increase of the utilization of herbal medications in the field of toxicology and pharmacology. The use of medicinal plants provides several advantages over the use of synthetic products, including a lower level of toxicity, fewer adverse side effects and multiple means of efficacy. Traditional reproductive health methods (e.g., conventional medical treatment, nutritional supplements) are generally available to manage infertility and hormone imbalance due to environmental and pharmaceutical issues (Singh and colleagues, 2020). Wet dried herbal formulations do not always achieve the same effects due to variability in dosages, treatment durations, and extraction methods. The standardization of herbal extracts along with identifying their active components will support more reliable therapeutic outcomes. Additional studies should be performed on how this plant affects the molecular pathways that underlie its protective properties, while clinical research will validate its safety and effectiveness. To summarize, this study has proved that cyproterone acetate produces serious toxicity through hormonal supression and oxidative stress, whereas *Tribulus terrestris* has demonstrated its ability to mitigate these toxicities by elevating testosterone levels, enhancing sperm formation and function, and decreasing oxidative damage. Therefore, this research supports the use of this plant as a natural agent to help protect individuals from the reproductive toxicities associated with pharmaceutical drugs.

Conclusion

In the current study, the results indicate that cyproterone acetate (CA) has considerable reproductive and endocrine toxicity within albino rats ([A] decreased sperm parameters, [B] hormonal imbalance, and [C] increased oxidative stress). *Tribulus terrestris* (TT) exhibited a significant protective effect in restoring reproductive function, improving metabolic hormone levels, and enhancing the antioxidant defense system. Therefore, TT may have potential as a natural therapy for alleviating drug-induced reproductive toxicity. Future research warrants further investigation into the clinical applications.

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