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Ethanolic Extract of *Capparis cartilaginea* Decne as an Ameliorative Agent Against CCl_4 Induced Testicular Damage in Male Albino Rats

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Abstract:
Capparis cartilaginea Decne. (Capparaceae) has been traditionally used for various therapeutic purposes. This study aimed to evaluate the ameliorative effect of *C. cartilaginea* ethanolic leaf extract on testicular damage induced by carbon tetrachloride (CCl_4) in rats. Twenty-five adult male albino rats, weighing between 130 and 180 g, were randomly divided into five groups. Group I (Control) received no treatment - Group TI was administered olive oil (0.5 ml orally) as a vehicle control - Group TII received the ethanolic leaf extract of *C. cartilaginea* at a dose of 500 mg/kg orally - Group TIII was treated with carbon tetrachloride (CCl_4) at a dose of 2 mg/kg, administered intraperitoneally on alternate days to induce testicular toxicity - Group TIV received CCl_4 (2 mg/kg intraperitoneally on alternate days) followed by ethanolic extract (500 mg/kg orally). At the end of the experiment, all rats were sacrificed and testes were collected for histopathological analysis. The CCl_4 treated group exhibited a significant reduction in both body weight and relative testicular weight, along with causing fibrosis, congestion, interstitial edema, and degeneration of the seminiferous tubules. In contrast, the control, TI, and all *C. cartilaginea*-treated groups, including TIV, which received CCl_4 followed by the ethanolic extract, preserved normal testicular architecture, similar to that of the control group. These results suggest that the ethanolic leaf extract of *C. cartilaginea* offers a restorative effect against CCl_4 induced testicular toxicity in rats.

INTRODUCTION

Medicinal plants have been used since ancient times as therapeutic agents due to their bioactive compounds, such as alkaloids, flavonoids, phenols, and terpenoids, which support health management and disease treatment (Ashraf *et al.,* 2020; Iqbal and Ashraf, 2018; 2023; Iqbal and Khalid, 2023). Herbal medicine remains the primary source of healthcare for approximately 75–80% of the global population, with a significant portion of traditional therapies relying on plant extracts and their active constituents (Acharya *et al.*, 2008; Zaynab *et al.,* 2018). The Capparaceae family includes 45 genera and over 700 species, comprising shrubs, trees, and woody climbers, mainly found in warm regions. Among them is *Capparis cartilaginea* Decne., which is a climbing shrub native to North Africa, Western Asia, India, and the Arabian Peninsula (Hamed *et al.,* 2007; Alsharif *et al.*, 2022; Alsharif and Boylan, 2025).

In traditional Arabian medicine, *C. cartilaginea* is employed to treat inflammation, earaches, headaches, bruises, snakebites, skin and joint disorders, as well as issues related to childbirth (Miller *et al.*, 1988; Phondani and Bhatt, 2016; Moharram and Al-Mahbashi, 2018). The plant is also used in pharmaceuticals (including drugs and cosmetics), food, and in animal nutrition (Al-Mahweety and Alyahawi, 2020). In Yemen, it is known locally as lattssaf, laşaf, or nişaf and is used for managing itching, shortness of breath, colds, tumors, wounds, and knee pain (Alzweiri *et al.*, 2011; Lansky *et al.*, 2013; Al-Qudah *et al.*, 2018; Alsharif *et al.*, 2022). Previous studies have indicated that *C. cartilaginea* exhibits various biological activities, including antioxidant, antimicrobial, antihyperglycemic, hypolipidemic, analgesic, anti-osteoporotic, larvicidal, hypotensive, and bradycardiac effects (Abutaha and Al-Mekhlafi, 2014; Rahimifard and Shojaii, 2015; Al-Balwi, 2018; Eddouks *et al.*, 2025). Moreover, phytochemical studies revealed that its leaves contain numerous active components, including carbohydrates, saponins, polyphenols (flavonoids and tannins), triterpenes, sterols, amino acids, isothiocyanates, and proteins (Gilani and Aftab, 1994; Sharaf *et al*., 1997; Mothana *et al.*, 2009; Galib and Algfri, 2016; Moharram and Al-Mahbashi, 2018).

Carbon tetrachloride (CCl_4) is an environmental contaminant still used in various industrial and domestic applications, including as an insecticide, in dry-cleaning processes, grain fumigation, and fire extinguisher formulations (Abd-Elhakim *et al*., 2020). In addition, CCl_4 serves as an industrial solvent and exhibits significant toxicity to multiple organs, including the kidneys, brain, heart, lungs, liver, and testes, compromising the structural and functional integrity of the male reproductive system through mechanisms involving oxidative stress (Abraham *et al.*, 1999; Ganie *et al.*, 2011). Studies with antioxidants confirm the central role of oxidative stress in CCl_4 induced testicular damage (Al-Olayan *et al.*, 2014). The toxic effects of CCl_4 are primarily attributed to its metabolic activation by the cytochrome P450 (CYP) enzyme system, which converts CCl_4 into highly reactive free radicals, including the trichloromethyl radical ($CCl_3^{\bullet}$) and/or trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$). These radicals induce lipid peroxidation and disrupt cellular membranes, leading to oxidative damage. Moreover, CYP enzymes, including both steroidogenic and non-steroidogenic isoforms, are expressed in reproductive tissues, suggesting a direct role in mediating CCl_4 induced testicular toxicity (Gilibili *et al.*, 2014). Several natural products rich in antioxidant compounds have also been reported to protect testicular tissue against lipid peroxidation and oxidative imbalance caused by CCl_4 exposure (Khan and Ahmed, 2009; Cemek *et al.*, 2010).

Therefore, the present study aims to investigate the ameliorative effect of the ethanolic extract of *C. cartilaginea* Decne against CCl_4 induced testicular damage in rats.

MATERIALS AND METHODS

Plant material

Plant material including specimens and leaf samples of *Capparis cartilaginea*, was collected from Al-Radma, Ibb Governorate, Yemen. The specimens were carefully examined and identified by Professor H. M. Ibrahim at the Faculty of Science Herbarium, Sana'a University, where a voucher specimen (BHSS 49) was deposited for future reference. The leaves were washed under running tap water, rinsed with distilled water, air-dried at room temperature, and ground into a fine powder for subsequent extraction.

Preparation of plant extract

The powdered plant material was extracted with 80% ethanol using the soaking method for 72 hours. The mixture was then filtered through Whatman No. 1 filter paper. The solvent was evaporated under reduced pressure using a rotary evaporator, then dried using a hot air oven (DHG-9202-ISA) at 40–45 °C for 7–8 hours. The resulting crude extract was desiccated, collected, weighed, and stored in dark glass containers at –4 °C for further analysis (Bennour *et al*., 2020).

Assessment of antioxidant activity via 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

The antioxidant activity of leaf extract (µg/ml) was assessed following the methodology described by Mansor *et al.* (2001). The evaluation focused on the extract's capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. This analysis was conducted at the National Center for Public Health Laboratories (NCPHL) in Sana'a.

The percentage of inhibition was determined according to Ibrahim *et al.* (2023) using the formula:

% inhibition = Ac – As/Ac × 100, where Ac = absorbance of the control and As = absorbance of the test sample (ascorbic acid and both extracts).

Ethical approval

The study protocol was approved by the Animal Ethics Committee of the Biological Science Department, Sana'a University (ethical code: BAHSS101).

Experimental animals

Twenty-five adult male albino rats weighing 130–180 g were used in this experiment. The animals were obtained from the Animal House, Department of Biology, Faculty of Science, Sana'a University, Yemen. Throughout the experimental period, the rats were housed in plastic cages under standard laboratory conditions (room temperature, 12 h light/dark cycle) and provided with standard food pellets and water ad libitum.

Experimental design

The animals were randomly assigned to five groups of five animals each. The control group (Group I) received a standard diet and water, whereas Group TI was administered olive oil (0.5 mL orally) as a vehicle control. Group TII was treated orally with *C. cartilaginea* ethanolic leaf extract (500 mg\kg) daily for 2 months, while Group TIII was carbon tetrachloride (CCl_4) at a dose of 2 mg/kg, administered intraperitoneally on alternate days to induce testicular toxicity for 2 months. Finally, Group TIV received CCl_4 (2 mg/kg intraperitoneally on alternate days) followed by ethanolic extract (500 mg/kg orally).

Body weight and relative testes weight

The body weights of rats in each of the five groups were recorded twice weekly throughout the treatment period to monitor weight changes. At the end of the experiment, the animals were euthanized, and their testes were excised and weighed. Relative testicular weight was calculated using the following formula as suggested by Udroiu *et al.* (2015):

Relative testicular weight (%) = (Testis weight / Body weight) ×100

Histology of the testes

For histopathological examination, testes from each experimental group were collected, and appropriately sized sections were fixed in 10% neutral-buffered formalin. The fixed tissues were then dehydrated using ascending grades of ethyl alcohol, cleared in xylene, and embedded in paraffin wax at 58°C. Serial sections (3 µm thick) were prepared using a rotary microtome. The sections were stained with Harris hematoxylin and eosin (H&E) and examined microscopically for histological alterations of the testes (Bancroft and Gamble, 2008; El-Sayyad *et al.*, 2009).

Statistical analysis

The data were expressed as mean and analyzed using one-way analysis of variance (ANOVA) with GraphPad Prism software, version 9.1.1. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Assessment of antioxidant activity via 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

The ethanolic leaf extract of *C. cartilaginea* exhibited notable antioxidant activity compared to ascorbic acid (a standard antioxidant). The antioxidant potential was assessed using the DPPH free radical scavenging assay, and the results were expressed as a percentage of inhibition (Table 1). The extract showed markedly greater inhibition values at higher concentrations, indicating a strong antioxidant potential.

Table 1. Comparative % inhibition of DPPH shown by ascorbic acid and C. *cartilaginea* ethanolic extract.

Extract	Concentration (µg/ml)	Scavenging activity (%)
Ascorbic acid	3	90
C. cartilaginea	15	80

Body weight and relative Testes weight

Administration of olive oil (Group TI), ethanolic plant extract (Group TII), and Group TIV, which received CCl_4 (2 mg/kg, intraperitoneally on alternate days) followed by ethanolic extract (500 mg/kg, orally), did not produce any significant change in average body weight compared with the control group. In contrast, Group TIII, which received CCl_4 alone (2 mg/kg, intraperitoneally), showed a significant reduction in body weight ($P < 0.0001$). Similarly, the relative testes weight was significantly decreased in the CCl_4 treated group compared with that in the control group ($P < 0.0001$). In contrast, Groups TI, TII, and TIV showed no significant differences in relative testicular weight compared with the control group, indicating that the ethanolic extract ameliorated CCl_4 induced testicular atrophy (Table 2).

Table 2. Effect of ethanolic extract of *C. cartilaginea* against CCl_4 on body weight and testes weight of rats.

Study groups	Effect of ethanolic extract of *C. cartilaginea* on weight gain		
	Body weight (g)	Testes weight (g)	Relative testis weight (%)
Control	361	6.24	1.73
Group TI	357.2	4.2	1.18
Group TII	355.4	5.61	1.6
Group TIII	258	7.72	3
Group TIV	334.7	5.62	1.7

Histological Alteration of Testes

Histological examination of the testes in the control group and group TI revealed normal seminiferous tubule architecture, separated by interstitial tissue. The seminiferous tubules were lined with a germinal epithelium containing spermatogonia and pyramidal Sertoli cells. Blood vessels and Leydig cells were observed within the interstitial tissue, and the tubular lumen contained eosinophilic threads resembling spermatozoa (Plates 1 and 2).

However, Group TII (treated with the ethanolic extract of *C. cartilaginea*) showed mild histological changes in the testes (Plate 3).

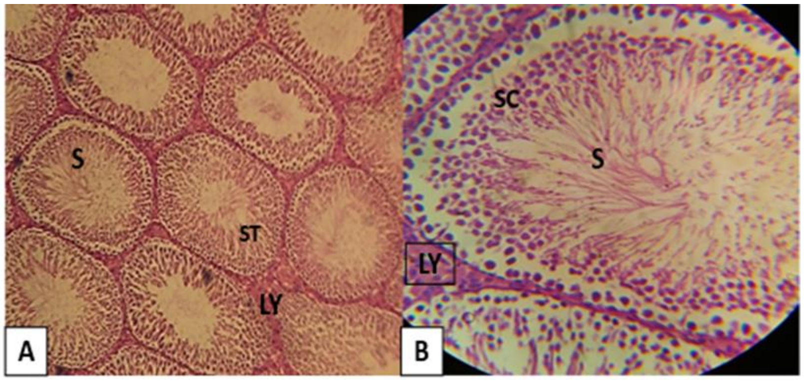


Plate 1 (A&B). The control group (C) shows seminiferous tubules (ST) separated by interstitial tissue that contains Leydig cells (Ly) and blood vessels. Sperm tails (S) seen as luminal eosinophilic threads. Germinal epithelium lining seminiferous tubules (ST) near the basement membrane pyramidal Sertoli cells (SC).

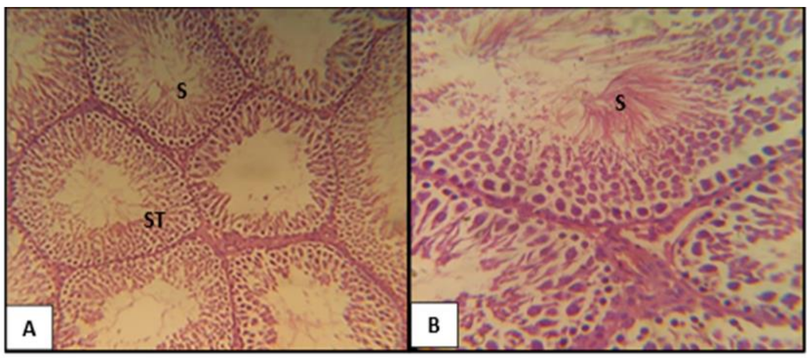
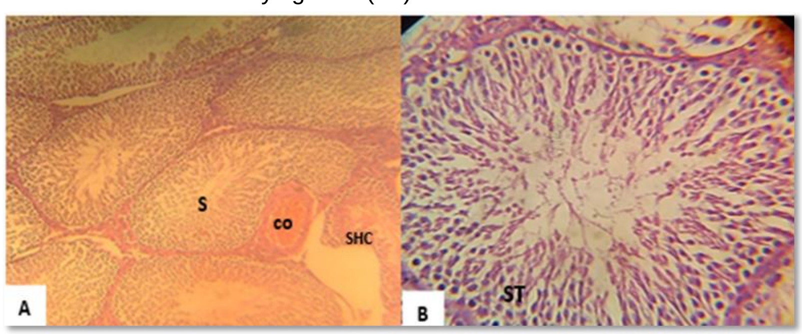


Plate 2(A&B). Histopathological photograph of TI group showed normal structure Seminiferous tubules (ST), Sperm tails (S) and interstitial tissue that contains Leydig cells (LY).



Palate 3 (A&B). Histopathological photograph of TII group showed normal Seminiferous tubules (ST) and Sperms (S), Congested blood vessels (CO), and shrinkage Seminiferous tubules (SHC).

Furthermore, Group TIII (received CCl_4) testicular sections exhibited pronounced histopathological alterations, including fibrosis, marked congestion of interstitial blood vessels, interstitial edema appearing as faint eosinophilic material, degenerative changes in the germinal epithelium, and cell shedding into the lumen. Moreover, additional alterations, including fatty changes, necrotic areas, and further degeneration of the seminiferous epithelium, were observed (Plate 4).

In contrast, in Group TIV (received CCl_4 followed by 500 mg/kg of the ethanolic extract of *C. cartilaginea*), the testes displayed a near-normal histological appearance, indicative of amelioration, although some edema was still observed (Plate 5).

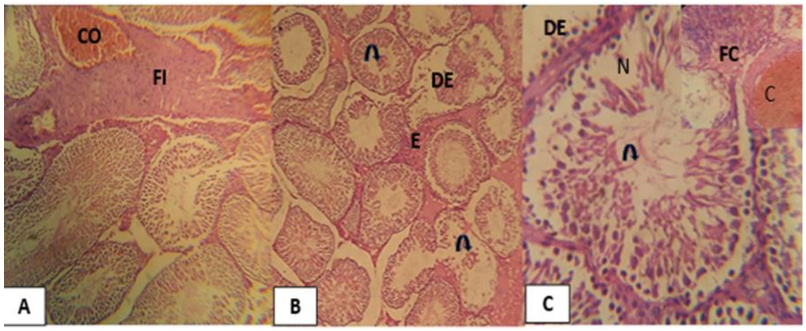


Plate 4 (A&B): Histopathological photographs of TIII group (CCL_4) administered group showing; **A:** Fibrosis (FI) and marked congestion of interstitial blood vessel (CO). **B:** Showing interstitial edema as a faint eosinophilic material (E), increasing degenerative changes in the germinal lining of Seminiferous tubules and shedding into the lumen (Curved arrows), Congestion (C). **C:** Fatty changes (FC), Necrotic area (N), degenerative changes in the germinal lining of Seminiferous tubules (DE), and Congested blood vessels (CO).

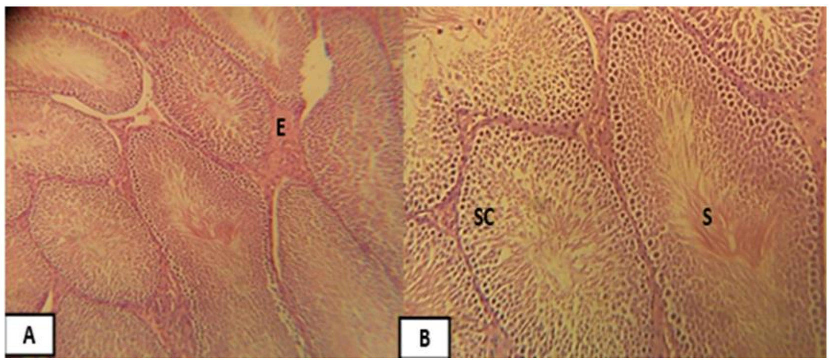


Plate 5 (A&B): Histopathological photographs of TIV group showing; **A:** Normal structure of Seminiferous tubules and interstitial edema (E). **B:** Normal structure of Seminiferous tubules (SC) and Sperms (S).

DISCUSSION

Exposure to environmental chemicals can negatively affect male fertility in several ways. A variety of harmful chemical substances are produced during industrial operations, including CCl_4, which can accumulate in the body and cause reproductive dysfunction (Farooq, 2005; Nowicka-Bauer and Nixon, 2020; Unsal *et al.,* 2021).

Based on the present results, *C. cartilaginea* ethanolic leaf extract showed strong antioxidant potential, which agrees with the findings of Thamer *et al.* (2024), who reported that *C. cartilaginea* extract contains bioactive compounds, such as phenolics, fatty acids, and carotenes, which contribute to its antioxidant activity.

According to the present results, group TIII, which was treated with CCl_4 (2 mg/kg), showed a significant decrease in body weight compared to the controls, likely due to reduced food intake, which aligns with previous studies reporting weight loss after CCl_4 exposure (Alkreathy *et al.*, 2014; El-Faras *et al.*, 2016).

Moreover, histological examination of group III revealed that CCl_4 caused degeneration of the seminiferous tubules, disruption of normal spermatogenesis, loss of spermatozoa, and damage to the testicular epithelium. These findings are consistent with earlier reports of CCl_4 induced testicular damage, including necrosis, fatty changes, and alterations in tubular structure (Rahmoni *et al.*, 2018; Keshtmand *et al.*, 2021; Eljaafari *et al.*, 2024). Furthermore, the present study did not observe significant changes in tubule diameter or cavity size.

The reduction in testicular damage in group IV (treated with 2 mg of CCl_4 followed by treatment with 500 mg/kg of the ethanolic leaf extract of *C. cartilaginea*) can be attributed to the strong antioxidant potential of *C. cartilaginea*. This finding agrees with the conclusions of Hashem (2021) and Bayramova et al. (2024), who reported that natural antioxidants, such as propolis and curcumin, can protect against and reduce testicular damage caused by CCl_4 induced testicular injury by counteracting oxidative stress and cell damage.

CONCLUSION

In conclusion, the ethanolic leaf extract of *Capparis cartilaginea* markedly improved testicular damage induced by CCl_4 in rats by restoring normal testicular architecture and improving seminiferous tubule integrity. These findings suggest that *C. cartilaginea*, with its strong antioxidant potential, may facilitate testicular recovery following chemical-induced injury, likely through the action of its bioactive and regenerative phytoconstituents.

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CONFLICT OF INTEREST

There is no conflict of interest.

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