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# Antifertility effect of methanolic leaf extract of *Chenopodium ambrosioides* Hook. in male Sprague Dawley rats

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## Abstract

This study evaluated the antifertility activity of methanolic extract of *Chenopodium ambrosioides* leaf on male rats. During the experiment, different doses of extract (0, 50, 100 and 150 mg/kg) were orally administered to rats for 28 days. Analysis of sperm parameters revealed a dose-dependent decrease in sperm motility, viability and daily sperm production (DSP). While, increased oxidative stress in reproductive organs; and impaired testicular and epididymal histology was also evident in high dose regimen. Furthermore, a reduction in concentrations of plasma testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) was also recorded. Reduced pregnancy outcome and small litter size in the females paired with treated male rats after 30 days of treatment withdrawn was noted in higher doses. From these findings, it is concluded that the methanolic leaf extract of *C. ambrosioides* is quite effective in reversible suppression of male fertility.

**KEYWORDS**

*Chenopodium ambrosioides*, contraception, histology, hormonal analysis, spermicide

## 1 | INTRODUCTION

World population has been tremendously increasing day by day with its undesirable consequences on environmental and economic growth in poor-developed countries (Thakur, Kumar, Kujur, Kumar, & Kumar, 2010). This situation would intensify the need for effective measures to control the birth rate. Afterwards, several attempts have been made to control the birth rate by various means. At the beginning, several synthetic compounds (Chemical nature) were used as oral contraceptives and very little attention was paid to the plant kingdom. However, use of these synthetic agents was associated with several deleterious side effects such as hormonal imbalance (Van der Vange, Blankenstein, Kloosterboer, Haspels, & Thijssen, 1990), increased risk of cancer (Giannitrapani, Soresi, Spada, Cervello, & D'alessandro, N. & Montalto, G., 2006),hypertension (Dubey, Oparil, Imthurn, & Jackson, 2002) and weight gain (McNamara, 2001). Therefore, there is a necessity to replace these substances by safe and effective alternative like plant-based contraceptive agents. Now a day, almost 50% of medicinal prescriptions constitute active principle (s) derived from plants (Pan et al., 2013). Therefore, medicinal plants hold a great promise for the discovery of new antifertility agents.

Several medicinal plants have antifertility properties (Garg, Talwar, & Upadhyay, 1998; Hiremath, Badani, Hunasagatta, & Patil, 2000; Hiremath, Rudresh, Badani, Patil, & Patil, 1999). Various herbal remedies are traditionally used as contraceptives abortifacients and emmenagogue agents (Ritchie, 2001). Numerous plants containing flavonoids, tannins, terpenes, quinines and diterpenoid lactones have been shown to induce male antifertility through different mechanism (Joshi, Sharma, & Chaturvedi, 2011; Rao, Vimalamma, Rao, & Tzeng, 2004; Reddy et al., 2003). Alkaloids, flavonoids, steroids, saponins and phenols found in Chenopodiaceae plants have been identified (Ibrahim et al., 2007; Kokanova-Nedialkova, Nedialkov, & Nikolov, 2009).

Chenopodium is a genus of numerous perennial or annual herbaceous flowering plants species which are cosmopolitan in distribution (Gelin, Sergei, & Steven, 2003). *C. ambrosioides* is a perennial, aromatic herbaceous shrub of family Chenopodiaceae that is extensively cultivated in Eastern and Central USA, Europe, Maryland, Mexico and Canada. Common name of *C. ambrosioides* is "mastruz"

  

or "erva-deSanta-Maria" in Brazil, whereas in other American countries it is commonly known as goosefoot, epazote and paico (Cruz et al., 2007; Ososki et al., 2002). Decoctions and infusions of inflorescences, roots and leaves of *C. ambrosioides* have been used for many centuries as a dietary condiment and in folk medicine.

Because *C. ambrosioides* is plentiful in flavonoids and terpenoids compounds, it is pharmacologically used as cancer chemopreventive agent (Kiuchi et al., 2002; Liu, 2004). Other therapeutic roles of *C. ambrosioides* include anthelmintic (Giove, 1996; Kliks, 1985), antiviral (Zanon, Ceriatti, Rovera, Sabini, & Ramos, 1999), antibacterial (Sousa et al., 2012), antifungal (Pandey, Singh, Palni, & Tripathi, 2013), antipyretic (Bum et al., 2011) and analgesic applications. They are widely used for the treatment of several metabolic disorders such as diabetes, hypercholesterolaemia along with other digestive, urogenital, respiratory and nervous disorders (De Pascual, Torres, & Perez, 1980). Few cases of intoxication have been studied in human beings and rats species which is accompanied with the consumption of essential oil of *C. ambrosioides* in fairly large amounts (Chevallier, 1996; Ruffa et al., 2002). These toxic effects are more likely due to the presence of terpenoids that despite of diverse pharmacological properties, also have toxic aspects (Kiuchi et al., 2002; Liu, 2004). Moreover, *C. ambrosioides* is also toxic for several insects and potentially used as a botanical insecticide (Gadano, Gurni, Lopez, Ferraro, & Carballo, 2002; Tavares & Vendramin, 2005). The most bioactive component of *C. ambrosioides* in this aspect is the ascaridole, an essential oil which is a well-known worm repellent substance (Pandey, Palni, & Tripathi, 2014). It was previously shown that aqueous extract of *C. ambrosioides* negatively influences the reproduction in *Drosophila melanogaster* (Wohlenberg & Silva, 2009).

However, no study yet was found in literature about the reproductive toxicity of this preparation in males. Considering this aspect, the aim of this study was to investigate if the aqueous extract obtained from fresh leaves of *C. ambrosioides* is able to induce antifertility effects and cause reversible reproductive impairment in male rats.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant material and extraction

#### 2.1.1 | Plant material

*Chenopodium ambrosioides* L. locally known as "Jungli Bathua" is herb found in many regions of Pakistan including Shangla Alpuria, Shahpur, Lilowni, Chakesar, Hayatabad, Ajaori, Attock.

Swat, Parachinar and Abbottabad (Hazara). Plant was obtained from agricultural and cultivated fields of District Abbottabad (Hazara), Pakistan at elevation of 1,256 m. It was identified by Dr. Mushtaq Ahmad at Plant Systematics and Biodiversity Lab and sample was deposited in herbarium under association number # 14226 of Pakistan. Experiment was conducted in the start of 2017 at Quaid-i-Azam University Islamabad.

#### 2.1.2 | Plant extract preparation

Leaves of *C. ambrosioides* were separated from stem and air-dried. These air-dried leaves, weighing almost 2 kg were stored until the preparation of the extract. Leaves were ground in waring blender and then sieved. The dried powder of leaves was extracted with ethanol (leaves to solvent ratio 1:10). The extracts were filtered using Whatman filter paper and concentrated on a rotary evaporator (Gulfraz, Qadir, Nosheen, & Parveen, 2007).

### 2.2 | Animals

Adult male Sprague Dawley rats (90 days old) were obtained from Primate Facility of animal sciences department, Quaide-i-Azam University Islamabad. Animals were housed in well ventilated room with 12 hr light/12 hr dark cycle and room temperature was maintained at 20–25˚C. They were provided with standard laboratory diet and tap water ad libitum. All the animals were randomised into various groups of ten animals per cage and acclimatised for one week before initiation of experiment. All the animal handling and procedures were approved by the ethical committee of animal sciences department.

### 2.3 | Experimental design

Experiment was designed to assess the effect of methanolic extract of *C. ambrosioides* leaves on reproductive functions of male rats. For this study, forty animals were divided into four groups having ten animals each ($n$ = 10). First group served as control and was provided with 0.9% saline via oral gavage. Animals in the remaining groups were treated with various doses of leaf extract (50, 100 and 150 mg/kg respectively). All the doses were administered orally for period of 28 consecutive days. Animals were monitored throughout experiment carefully to see any morphological or physical changes.

### 2.4 | Blood and tissue collection

On day twenty-ninth, seven animals from each group were weighed and sacrificed by decapitation. Trunk blood was drawn and kept in heparinised tubes. All the blood was centrifuged at 1008 g for 15 min, plasma was separated and stored at −20°C until hormonal and biochemical analysis. Tissues of reproductive tract (testis and epididymus) were dissected out; washed with saline and weighed. Left testis was kept in freezer for analysis of daily sperm production. Left epididymis was minced for further assessment of epididymal sperm count and other sperm parameters. Right testicular tissue and epididymis were fixed in 10% formalin for histological studies.

### 2.5 | Fertility test

At the end of experiment, three animals from each methanolic extract treated group were paired with two untreated fertility proven female

rats individually. Vaginal smear from each matted rat was prepared for three consecutive days and number of spermatozoa in the smear was counted. Their litter size, morbidity and mortality if any, were noted.

## 2.6 | Effect on Sperm Motility

For the assessment of sperm motility, small portion of cauda epididymis was cut and crushed in 1 ml of 37°C normal saline solution to make homogeneous mixture. 10 µl of homogenate sample was taken with help of pipette and placed on a prewarmed slide. A minimum of 10 fields were observed and 100 spermatozoa were calculated via high power microscopy at 40× magnification (Halvaei, Sadeghipour, & Naghibi, 2012).

## 2.7 | Evaluation of Sperm Viability

Eosin-nigrosin staining test was used for the assessment of sperm viability. Semen samples were mixed with dye (eosin-nigrosin). With the help of a pipette, 15 µl droplet of this mixture was placed on a glass slide. Smear was prepared and air-dried at room temperature. These slides were examined under a light microscope at 40× resolution. Alive spermatozoa remained unstained (white) whereas, dead cells were stained red. The percentage of dead and alive spermatozoa was calculated by counting at least 100 sperm cells (Halvaei et al., 2012).

## 2.8 | Epididymal Sperm Count

For the determination of epididymal sperm count, small portion of caput, corpus and cauda epididymis was cut, placed and crushed in 1 ml of 37°C normal saline solution. 2–3 drops of nigrosine stain was also added into this mixture. With the help of a pipette, 10 µl of this homogenate sample was taken and placed on a prewarmed glass slide. A minimum of 10 fields were observed and spermatozoa were counted in each part of epididymis through high power microscopy at 40× magnification.

## 2.9 | Daily Sperm Production (DSP)

Frozen testicular tissues of all animals were thawed at room temperature for few minutes before homogenisation. Tunica albuginea was removed; parenchyma was weighed and homogenised in 3 ml of 0.9% sodium chloride (NaCl) solution containing 0.5% triton X-100, followed by homogenisation for 30 s (Robb, Amann, & Killian, 1978). The homogenate thus prepared was diluted upto 5 folds. 20 µl of this homogenate sample was deposited on Neubauer chambers and late spermatids were counted under microscope at 40× magnification. Three readings were taken twice for each sample to calculate average number of spermatids. Later on, this reading was then used to assess the total number of all the spermatids per testis. For the calculation of DSP, the number of spermatids which were noneffective against homogenisation (per testis and per gram of testis) was divided by 6.3, which represents the number of days these spermatids resides in seminiferous epithelium.

## 2.10 | Antioxidant enzymes

The stored testicular samples were thawed and homogenised in 3 ml of phosphate buffer having pH 7.4. The homogenate was centrifuged at 100800 g for 30 min at 4°C. Supernatant was collected for determination of antioxidant status of testicular tissue.

Plasma antioxidants Catalase (CAT; Chance & Maehly, 1955), Superoxidase dismutase (SOD; Kakkar, Das, & Viswanathan, 1984), Peroxidase activity (POD; Chance & Maehly, 1955), Lipid peroxidation (TBARS; Wright, Colby, & Miles, 1981) and Reactive oxygen species (ROS; Hayashi et al., 2007) were estimated.

## 2.11 | Tissue histology

Testis and epididymis were fixed in 10% formalin for forty-eight hours followed by dehydration in alcoholic grades and clearing in xylene. After that, tissues were embedded in paraffin wax and blocks were prepared for microtomy. Transverse sections of five micrometre thickness were cut and every tenth section was placed on prewarmed albumenised slides. Air-dried and kept in oven at 37 °C overnight for complete deparafinisation. Sections were stained with Hematoxylene and Eosin (H&E) followed by rehydration in alcoholic grades. Three small drops/slide of DPX were added and then coverslip was carefully placed before the permount dried.

## 2.12 | Light microscopy

Prepared slides were observed under Leica Microscope (New York Microscope company) equipped with digital camera (Canon, Japan). Images were taken at 20 and 40 magnification. For histomorphometric analysis, following parameters were studied using image J2× software package programme; seminiferous tubular and luminal diameter, epithelial height of seminiferous tubule and thickness of tunica albuginea of testicular tissue while diameter of tubules, lumen and epithelial height of epididymal tissue were also studied. Number of different cell types was also counted from seminiferous tubules at 100× and mean number of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids in all treated groups were also reported.

## 2.13 | Hormonal analysis

Plasma concentrations of testosterone, leutinizing hormone (LH) and follicle stimulating hormone (FSH) were quantified using EIA kits (Biocheck Inc, USA), using manufacturer's instructions provided with the kit.

## 2.14 | Statistical analysis

Values were expressed as Mean ± *SEM*. Data was analysed by one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test (to compare different groups with control) using Graph Pad Prism 5 software. Level of significance was set at $p < 0.05$.

## 3 | RESULTS

### 3.1 | Gravimetric analysis

A significant increase in body weight was detected in animals of all the *C. ambrosioides* leaf extract treated groups in comparison with control group (Table 1). A prominent decrease ($p < 0.001$) in both right and left testicular weight was observed in 50 mg/kg dose treated group while no change was seen in other extract treated groups. In a similar manner, no significant change was seen in epididymal and prostate weight of all the extract treated groups. However, weight of seminal vesicle was reduced ($p < 0.01$) in 50 mg/kg group when compared to control.

### 3.2 | Fertility test

Effect of *C. ambrosioides* leaf extract on the fertility and mean number of pups born per female after 60 days of treatment withdrawal is given in Table 2. Low number of spermatozoa was observed in vaginal smear of untreated female rats mated with treated male rats. A dose-dependent decrease in percentage fertility and number of pups born per female were observed in all dose regimens when compared to control group. In group treated with 50 mg/kg methanolic extract, the fertility rate was 83%. However, in 100 mg/kg treated group, 66% of females were observed to be conceived while only 50% females conceived in 150 mg/kg leaf extract treated group. The no of pups born per female was also found to be decreased dose dependently in all treated groups as compared to control group. However, no signs of morbidity or mortality were observed in resultant pups.

### 3.3 | Sperm motility and viability

Mean ± *SEM* of sperm motility and viability rate of control and treated groups are shown in Table 3. A significant reduction ($p < 0.05$) in sperm motility rate was seen in dose-dependent manner. There was nonsignificant reduction in sperm viability rate observed in all the extract treated groups when compared to control group (Table 3).

### 3.4 | Epididymal sperm count

A significant reduction ($p < 0.01$) in caput sperm number was noticed in high and medium dose treated groups as compared to control. While nonsignificant decrease was seen in 50 mg/kg group. A significant decrease in the corpus sperm count was observed in 50 mg/kg ($p < 0.01$) and 150 mg/kg ($p < 0.001$) groups when compared to control. Cauda sperm count was significantly reduced ($p < 0.001$) in all the treated groups when compared to control group as shown in Figure 1.

**TABLE 1** Effect of *Chenopodium ambrosides* leaf extract on mean ± *SEM* body weight gain (g) of control and treated adult male rats

| Groups ($n = 10$) | Body weight gain (g) |
|---|---|
| Control | 80.2 ± 6.92 |
| 50 mg/kg | 51.4 ± 10.13 |
| 100 mg/kg | 74 ± 7.22 |
| 150 mg/kg | 58.02 ± 8.37 |

**TABLE 2** Effect of *Chenopodium ambrosioides* leaf extract on the fertility and the Mean ± *SEM* number of pups born per female after 60 days of treatment withdrawal

| Groups | Per cent fertility | Number of pups born/female |
|---|---|---|
| Control | 100 | 8.5 ± 0.83 |
| 50 mg/kg | 83 | 6.16 ± 1.76 |
| 100 mg/kg | 66 | 5 ± 1.77 |
| 150 mg/kg | 50 | 3.16 ± 1.55* |

*Notes*. Values are expressed as mean ± SEM
*,**,***Significant difference at probability $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control (ANOVA followed by Dunnet's multiple comparison test).

**TABLE 3** The mean ± *SEM* of % sperm motility and viability rate among control and treated groups of adult male rats after 28 days of experiment

| Groups | Motility rate (%) | Viability rate (%) | Daily sperm production × $10^6$/testis |
|---|---|---|---|
| Control | 75.50 ± 2.25 | 80.00 ± 7.07 | 2.47 ± 0.19 |
| 50 mg/kg | 72.50 ± 4.79 | 76.50 ± 4.19 | 1.20 ± 0.14*** |
| 100 mg/kg | 50.00 ± 5.77* | 62.50 ± 4.79 | 0.79 ± 0.10*** |
| 150 mg/kg | 55.00 ± 6.45* | 60.00 ± 7.07 | 0.58 ± 0.06*** |

*Notes*. Values are expressed as mean ± *SEM*
*,**,***Significant difference at probability $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control (ANOVA followed by Dunnet's multiple comparison test).

### 3.5 | Daily sperm production

Mean ± *SEM* of daily sperm production in control and treated groups are shown in Table 3. A dose-dependent decrease ($p < 0.001$) in daily sperm production was observed in all extract treated groups.

### 3.6 | Antioxidant enzymes

Exposure to methanolic extract of chenopodium resulted in significant decrease ($p < 0.001$) in catalase activity within testis of all the treated groups when compared to control group. In a similar manner, the activity of SOD was decreased significantly ($p < 0.05$) in 150 mg/kg group when compared to that of control group. While nonsignificant change was seen when comparison was made between low dose treated and control group. Peroxidase activity was found to be significantly reduced ($p < 0.001$) in 100 mg/kg and 150 mg/kg groups as compared to control group, whereas no significant change was observed in 50 mg/kg group (Table 4). The value of TBARS was increased significantly ($p < 0.01$) in 150 mg/kg group as compared to control while no significant change was seen in all other treated groups.

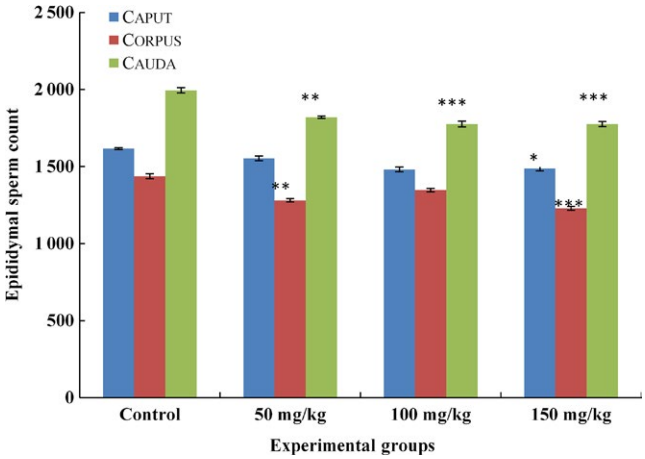


**FIGURE 1** Mean ± *SEM* of epididymal sperm production in control and treated groups of adult male rats after 28 days of treatment. *Notes*. *,**,***Significant difference at probability $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control (ANOVA followed by Dunnet's multiple comparison test).

In all the extract treated groups, an increase in ROS value was observed in comparison with control group, shown in Table 4. A significant increase ($p < 0.05$) in ROS value was seen in 150 mg/kg group while other treated groups showed nonsignificant change in ROS value when comparison was made with control group.

## 3.7 | Histomorphometric analysis

Histomorphometric findings of testicular and epididymal tissues are given in Table 5 and 6. The diameter of seminiferous tubule and lumen, epithelial height and tallness of tunica albugenia of testicular tissues were considered, whereas, in case of epididymis, diameter of lumen and duct along with height of epithelium was considered.

### 3.7.1 | Testis

No significant change was detected in lumen diameter of 50 mg/kg and 100 mg/kg treated groups when comparison was made with control group. However, significant increase ($p < 0.05$) was seen in 150 mg/kg extract treated group. Epithelial height was found to be little effected by dose administration. However, irregular arrangement of seminiferous tubules with interstitial spaces and low number of spermatozoa in epithelium were observed in higher dose regimens as shown in Figure 2. The unremarkable change was seen among low and medium dose treated groups. There was a substantial decrease ($p < 0.01$) in tunica albuginea height in 150 mg/kg dose treated group in comparison with the control one.

### 3.7.2 | Epididymis

Table 6 shows histomorphometric results for caput and cauda epididymis.

#### Caput epididymis

No noteworthy change was observed in ductular diameter of 50 and 150 mg/kg dose treated groups as compared to control. However, remarkable decrease ($p < 0.05$) was seen in 100 mg/kg extract treated group. A little change in luminal diameter was observed in all dose treated group as compared to control group, although, the change was not significant as shown in Figure 3. The epithelial height was found to be reduced in dose-dependent manner in all the dose treated groups. A significant change was evident in 100 mg/kg and 150 mg/kg dose treated groups.

**TABLE 4** Effect of *Chenopodium ambrosides* leaf extract on mean ± *SEM* antioxidant activity of testicular tissues in experimental groups

| Groups | CAT (U/mg) | SOD (U/mg) | POD (nmole) | ROS (µmol/min) | TBARS (nM/mg) |
|---|---|---|---|---|---|
| Control | 41.30 ± 2.41 | 49.41 ± 4.45 | 5.88 ± 0.54 | 0.77 ± 0.23 | 2.01 ± 0.41 |
| 50 mg/kg | 31.02 ± 2.044** | 47.60 ± 6.29 | 4.16 ± 0.67 | 1.03 ± 0.02 | 2.35 ± 0.5 |
| 100 mg/kg | 11.83 ± 2.29*** | 33.205 ± 2.97 | 1.48 ± 0.44*** | 1.50 ± 0.39 | 3.47 ± 0.42 |
| 150 mg/kg | 5.52 ± 0.84*** | 31.49 ± 2.93* | 1.43 ± 0.46*** | 1.97 ± 0.26* | 4.59 ± 0.47** |

*Notes*. Values are expressed as mean ± *SEM*
*,**,***Significant difference at probability $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control (ANOVA followed by Dunnet's multiple comparison test).

**TABLE 5** Assessment of mean ± *SEM* of seminiferous tubule diameter, tubular lumen diameter, seminiferous tubule epithelial height and tunica albugenia height of testis in control and treated groups after 28 days of treatment

| Groups | Seminiferous tubule diameter (µm) | Tubular lumen diameter (µm) | Epithelial height (µm) | Tunica albugenia height (µm) |
|---|---|---|---|---|
| Control | 218.09 ± 7.23 | 33.10 ± 1.10 | 83.38 ± 2.01 | 26.36 ± 1.10 |
| 50 mg/kg | 212.02 ± 7.90 | 35.36 ± 1.05 | 78.48 ± 2.16 | 23.43 ± 0.86 |
| 100 mg/kg | 203.45 ± 5.80 | 40.15 ± 3.72 | 79.87 ± 3.56 | 21.37 ± 1.14 |
| 150 mg/kg | 211.71 ± 7.31 | 39.54 ± 2.25* | 75.25 ± 3.37 | 20.05 ± 0.80** |

*Notes*. Values are expressed as mean ± *SEM*
*,**,***Significant difference at probability $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control (ANOVA followed by Dunnet's multiple comparison test).

**TABLE 6** Mean ± *SEM* of ductular diameter (μm), luminal diameter (μm) and epididymis cell height (μm) of epididymis in control and treated groups after 28 days of treatment

| | Ductular diameter (μm) | | Lumen diameter (μm) | | Epithelial height (μm) | |
|---|---|---|---|---|---|---|
| Groups | Caput | Cauda | Caput | Cauda | Caput | Cauda |
| Control | 353.58 ± 8.14 | 451.44 ± 15.48 | 228.12 ± 19.47 | 410.78 ± 12.94 | 31.36 ± 2.30 | 36.90 ± 1.79 |
| 50 mg/kg | 323.93 ± 18.49 | 446.86 ± 9.44 | 226.65 ± 11.55 | 411.57 ± 9.01 | 26.70 ± 0.99 | 35.58 ± 1.74 |
| 100 mg/kg | 290.77 ± 11.92* | 447.40 ± 10.02 | 234.67 ± 8.77 | 399.18 ± 8.57 | 25.81 ± 1.04* | 30.33 ± 1.36* |
| 150 mg/kg | 273.07 ± 21.04 | 442.46 ± 15.10 | 235.70 ± 27.15 | 403.95 ± 13.80 | 21.45 ± 1.61* | 32.81 ± 2.28 |

*Notes*. Values are expressed as mean ± *SEM*
*,**,***Significant difference at probability $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control (ANOVA followed by Dunnet's multiple comparison test).

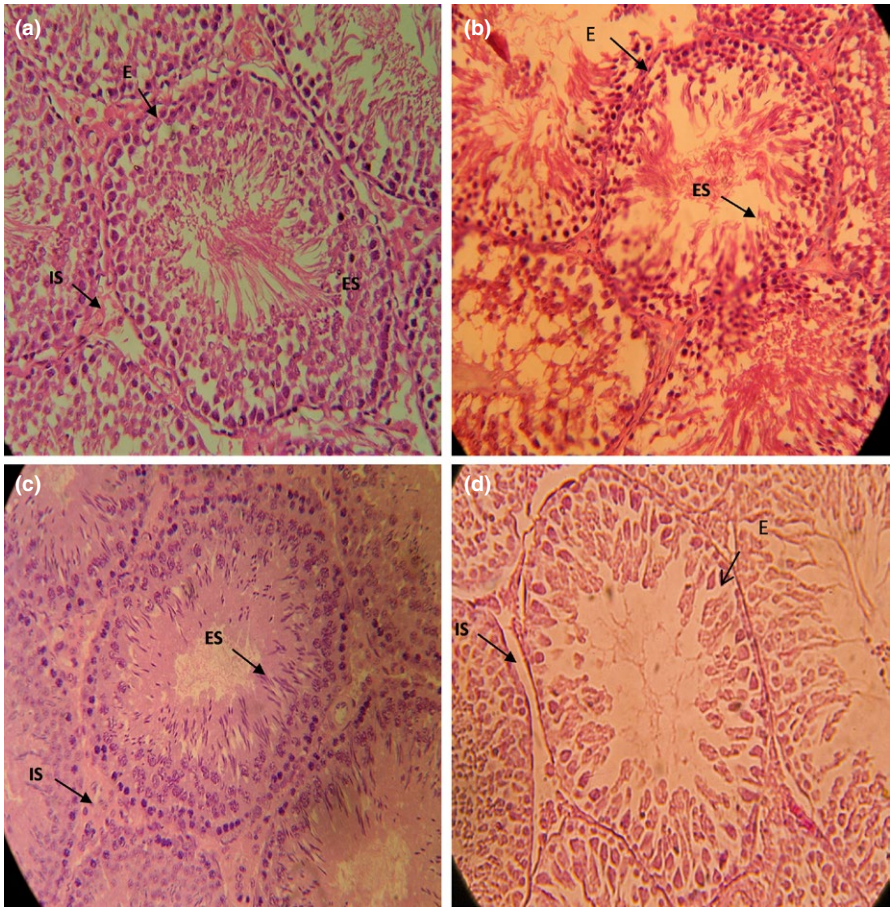


**FIGURE 2** Photomicrograph of seminiferous tubules. Regularly arranged tubules, lumen filled with spermatids with normal germ cells shown in (a) Control; represents normal process of spermatogenesis, lumen filled with mature spermatozoa, (b) low dose treated groups; showing tubules with immature spermatids released in lumen and degenerated epithelial layer, (c) medium dose treated group; showing reduced lumen diameter and amplified epithelial height, tubules with interstitial spaces are present, (d) high dose treated group; thin epithelium, lumen is empty, large interstitial space are present. Magnification ×40

### Cauda Epididymis

The ductular and lumen diameter of caudal epididymis was reduced in all the dose treated groups as compared to control group but the change was nonsignificant. Epithelial cell height of cauda epididymis was found to be significantly reduced ($p < 0.005$) in 100 mg/kg dose treated group as compared to control. No significant change was seen in low and high dose groups as presented in Figure 4.

## 3.8 | Hormonal analysis

The concentrations of plasma testosterone (ng/dl), luteinizing hormone and follicle stimulating hormone in adult male rats following 28 days of treatment has been given in Table 7. Plasma testosterone levels were reduced significantly ($p < 0.05$) in 50 mg/kg and 100 mg/kg dose treated groups when compared with control group. In a similar manner, a significant decrease ($p < 0.001$) was also observed in 150 mg/kg extract treated group when it was compared to control group.

Plasma LH concentration (IU) was decreased in all extract treated groups when compared to control group but this reduction was not significant. In a similar manner, plasma FSH levels were decreased in all extract treated groups when compared to control. A significant decrease in plasma FSH concentration (IU) was observed in 50 and 150 mg/kg extract treated rats ($p < 0.05$ and $p < 0.01$ respectively)

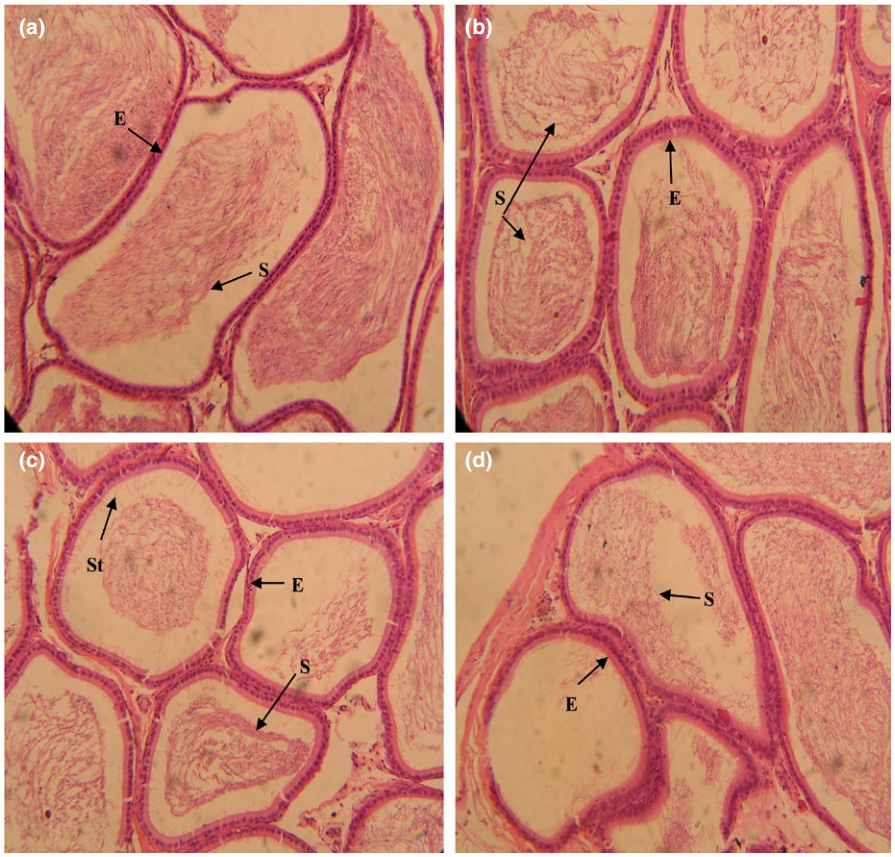


**FIGURE 3** Photomicrograph of adult male rat caput epididymis (H&E, 40×) from: (a) Control group; with normal morphology of caput epididymal cells having thin pseudostratified epithelium lined with stereocilia. Lumen is heavily filled with spermatozoa, (b) low dose treatment group; reduced pseudostratified epithelium with lumen filled with spermatozoa, (c) medium dose treatment group; showing reduction in number of spermatozoa, low number of spermatozoa in lumen and quite thick epithelium, (d) high dose treatment group; showing increase in pseudostratified epithelium and very little concentration of spermatozoa as compared to other two groups group. Stereocilia (St), Epithelium (E), Spermatozoa (S)

while nonsignificant change in 100 mg/kg dose treated groups was seen in comparison with control group.

## 4 | DISCUSSION

In a developing country, involvement of males in family planning is restricted due to lack of literacy, education and access to healthcare facilities. One basic aim of research is to develop herbal contraceptive drugs for men that are more suitable for protective motives and monetary reasons (Naik et al., 2016). Numerous therapeutic plants are known to own wide-ranging antifertility properties in male, usually affecting spermatogenesis/steroidogenesis in the testis (Patel, Singh, Singh, & Singh, 2017; Naik et al., 2016). The medicinal significance of wormseed or Mexican tea, *C. ambrosioides* is commonly acknowledged in various regions of the world where several studies have testified that the plant possess antileishmanial, antifungal, nematicidal, antischistosomial properties, antiaflatoxigenic and antioxidant activity (Bai, Liu, & Liu, 2011; Chekem et al., 2010; Moilo, Dorcas, Joseph, & Gerald, 2014; Monzote, Pastor, Scull, & Gille, 2014). Previously, no study has been carried out to reveal the contraceptive potential of this plant; therefore, the present studies were conducted and the results showed that methanolic leaf extract of *C. ambrosioides* exerts antifertility effects on male reproductive system of adult rats as depicted by significant reduction in fertility rate.

In present study, administration of *C. ambrosioides* leaf extract for 28 days in adult male rats induced highly significant reduction in sperm motility and viability among all the extract treated groups I, II, III (50, 100, 150 mg/kg) as compared to control group (0 mg/kg). Reduced fertility rate and pregnancy outcomes in females mated with treated males were also observed. Complete spermatogenic arrest is not necessary for male contraception; fertility can be eliminated by altering structure or function of spermatozoa (Dwivedi, Chaudhary, & Sarine, 1990). Many studies have related the plant-based male sterility with decreased sperm number and sperm motility (Thanga, Sakthidevi, Muthukumaraswamy, & Mohan, 2012; Wtche et al., 2001). The results were in line with studies of Ramya, Misro, Sinha and Nandan, 2010. This reduction might be due to capability of plant extract to cross blood–testis barrier (BTB) and either hinder with normal process of spermatogenesis in seminiferous tubules either by effecting sperm proteins and sertoli cells, altering

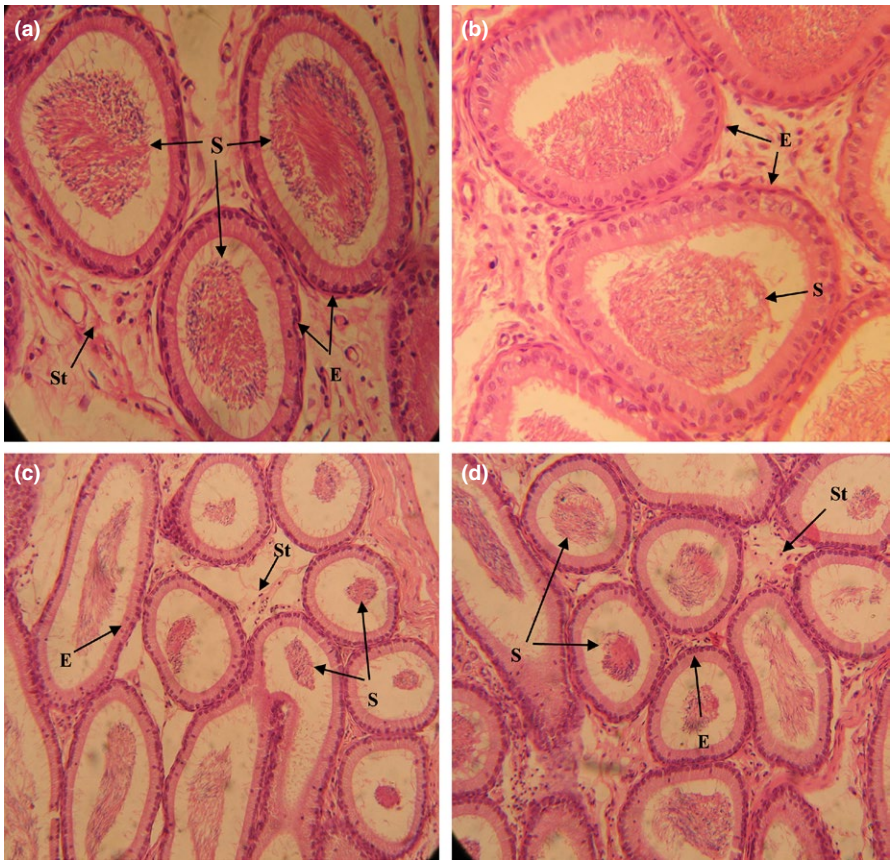


**FIGURE 4** Photomicrograph of cross section of cauda epididymis (H&E, 40×) male rats receiving doses of *C. ambrosoides* showing (a) Control; exhibiting normal morphology of cauda epididymis; compactly arranged tubules with thick epithelium, lumen filled with mature sperms, (b) low dose treated group; showing marked changes in structure of tubule with thick epithelium while lumen filled with sperms, (c) medium dose treated group; showing irregular arrangement of tubules surrounded by stroma, lumen has very little number of spermatozoa, (d) High dose treated group; showing further maximum increase in epithelial height and a little in lumen sperm concentration. Spermatozoa (S), Epithelium (E), Stroma (St)

**TABLE 7** Mean ± *SEM* plasma testosterone (ng/ml), LH (IU) and FSH (IU) concentrations in control and treated groups after 28 days of treatment

| Groups | Plasma Testosterone concentration (ng/ml) | Plasma LH concentration (IU) | Plasma FSH concentration (IU) |
|---|---|---|---|
| Control | 8.58 ± 0.39 | 1.53 ± 0.17 | 2.38 ± 0.16 |
| 50 mg/kg | 5.83 ± 0.92* | 1.06 ± 0.06 | 1.14 ± 0.32* |
| 100 mg/kg | 5.52 ± 0.35* | 1.25 ± 0.12 | 1.24 ± 0.40 |
| 150 mg/kg | 3.20 ± 0.61*** | 1.24 ± 0.20 | 0.47 ± 0.09** |

*Notes*. Values are expressed as mean ± *SEM*
*,**,***significant difference at probability $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control (ANOVA followed by Dunnet's multiple comparison test).

epididymal function and effecting motility of mature spermatozoa, or altering androgen synthesis and feedback regulation of HPG axis shifting spermatogenesis as suggested by Naik *et al.* (2016) in their respective study.

The sperm count and motility rate determine the fertility status of an individual (Ghosh, Jana, Pakhira, Tripathy, & Ghosh, 2015). A marked decline in epididymal sperm count was observed in dose-dependent manner in our study when adult male rats were exposed to varied doses (50, 100 and 150 mg/kg) of methanolic extract of *C. ambrosoides* for 28 days. Similar findings were also previously reported by Ramya and Shivanandappa, (2017) in their studies where treatment of male rats with methanol fruit extract (300, 900 mg/kg bw) for 60 days resulted in reduced sperm number in the epididymis (Ramya & Shivanandappa, 2017). A significant reduction in daily sperm production was also detected in all the extract treated (50, 100 and 150 mg/kg) groups as compared to control group. The exact mechanism by which the methanolic extracts decreased sperm count is not known, but it has been suggested that the plant components may have crossed the blood–testis barrier to exert detrimental effects on seminiferous tubules of the testes. Reduction in sperm count indicates alteration in normal steroidogenesis and disruption of morphological organisation in testes, compromising semen quality as suggested by Ghosh et al. (2015) in their respective work.

A balance between oxidants/antioxidants is necessary to be maintained to prevent body from oxidative stress and reproductive impairments (Ghosh et al., 2015). The current study presented that oral administration of adult male Sprague Dawley rats with different concentrations of *C. ambrosoides* leaf extract for 28 days of trial shifted normal oxidative/antioxidant status of body and induced production of reactive oxygen species (ROS).

The antioxidant enzyme activity was measured for two important enzymes, CAT and SOD whose levels were found significantly reduced in a dose-dependent manner in testes. Both are found to have free radical scavenging activity in male reproductive organs. POD is another enzyme involved in cell defence mechanism which converts hydrogen peroxide into water through oxidation-reduction reaction and prevents cell damage and death. Its levels were also significantly reduced in present study suggesting increased production of free radical byproduct such as TBARs and reactive oxygen species. The results of our study are in agreement with that of Ghosh et al. (2015) where increased level of free radical by-products such as CD and TBARS in the testicular tissues reinforced the generation of ROS, when Aqueous-ethanolic (1:1) extract of *T. chebula* fruit was orally administered at a dose of 60 mg/0.5 ml distilled water/day to adult fertile male rats for 28 days of experiment.

Histopathological studies of testes showed methanolic extract of *C. ambrosoides* caused a drastic and dose dependent reduction in number of spermatogonia, spermatocytes and mature spermatozoa as well as sloughing off of germinal epithelium in all the dose treated groups (50, 100 and 150 mg/kg). Seminiferous tubules were visible with empty lumen, degenerated epithelial layer and increased interstitial space in 50 mg/kg group with increased tubular diameter and wider lumen spaces. With increase in dose regime (100 mg/kg of plant extract) reduced lumen diameter and amplified epithelial height was evident. Here, compact tubules with little interstitial space were present. The high dose treated group (150 mg/kg) showed maximum damage to epithelium, empty lumen and large interstitial spaces present.

The development and maturation of spermatozoa together with normal process of steroidogenesis is the key to determine prime male fertility. Both events of spermatogenesis and steroidogenesis within testes are controlled by normal production and release of gonadotropins LH and FSH coming from anterior pituitary (Sreedhar Naik et al., 2016). Testosterone is produced from two sources, first from leydig cells as a result of positive induction with LH, secondly from medullary region of kidney. The appropriate levels of testosterone are necessary to carry out normal functions of testes. The present findings showed that methanolic leaf extract of *C. ambrosoides* affected the normal function of leydig cells resulting in significantly reduced production of plasma and intratesticular testosterone levels in a dose-dependent manner. Reduced testosterone concentrations are contributed by significantly reduced levels of LH in male rats thus encouraging spermatogenic arrest and infertility.

## 4.1 | Conclusion

Thus, it is concluded that methanolic leaf extract of *C. ambrosioides* caused partial male sterility by disturbing spermatogenic cycle, inducing oxidative stress and hormonal imbalance. Although, sperm motility and density were also reduced but fertility suppression was reversible after cessation of treatment. As no foetal mortality or morbidity was evident, so it is suggested that *C. ambrosioides* is capable to suppress male fertility without adverse toxicity.

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## DECLARATION OF INTEREST

The authors report no declarations of interest.

## AUTHOR CONTRIBUTION

The plant was collected, identified and extract was prepared by Mushtaq Ahmed. Sarwat Jahan led the experimental design and approved the study. Qurat-ul-Ain conceived the study, performed the experimental work, analysed the results and wrote the paper with input from other authors. Mehwish David and Qasim Shah performed initial part of the study, performed the experiment and helped in compiling the results.

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