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**Research Article**

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# Protective Effect of *Echinacea purpurea* Herb Extracts against Reproductive Toxicity in Male Rats

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**Abstract** Background: *Echinacea Purpurea* (family *Asteraceae*) is a perennial medicinal herb with important immunostimulatory, antioxidant, antibacterial and anti-inflammatory properties. Aim: The current study was carried out to investigate the protective effect of *Echinacea purpurea* (*E. purpurea*) ethanolic and aqueous extracts on male fertility in rats after induction of testicular damage by oral administration of Aluminium chloride ($AlCl_3$) for 60 days. Materials and Methods: Sixty-six male Wistar rats were randomly distributed into 6 equal groups (n= 11 rats). Group (1) was kept as a negative control given the vehicle and groups (2) and (3) were given either ethanolic or aqueous extracts of *E. purpurea* (150 mg/kg) alone for 60 days, respectively. Group (4) was given orally $AlCl_3$ (100 mg/kg) alone and used as a positive control group. Groups (5) and (6) were administered either ethanolic or aqueous extracts of *E. purpurea* + $AlCl_3$, respectively. Blood samples were withdrawn from orbital plexus of veins for estimating serum testosterone level. Semen samples were collected from cauda epididymis for examination of semen picture. Histopathological examination of testes was also carried out. Results: There were significant increases in sperm cell count, motility and viability as well as serum testosterone levels in *E. purpurea* extract-treated groups as compared to the positive control group. Testicular histopathological lesions were ameliorated in extract-treated groups. Conclusion: These results denote the beneficial effect of *E. purpurea* extracts on male fertility in rats. These findings confirm the use of *E. purpurea* herb in folk medicine for treating men with low fertilizing capacity.

**Keywords** *Echinacea purpurea*, Aluminum chloride, testosterone, sperm, histopathology

## Introduction

Aluminum (Al) is the most widely distributed metal on earth [1]. Humans exposed to Al through food, drinking water, and pharmaceutical preparations such as phosphate binders, antacid, buffered aspirins, buffered analgesics, antidiarrheal, antiulcer, cosmetics, antiperspirants, and vaccines with Al adjuvant [2]. Oral Al intoxication is mainly from the food itself, such as manufactured cheese and baking powders, or cookware such as cans[3]. After topical, oral, nasal exposure to Al, it binds to plasma transferrin and accumulated in muscle, bone, lung, and brain [3].

Testis and epididymis weight shrinkage, intratesticular edema, degenerated seminiferous tubules, abnormal spermatogenesis, altered sperm motility and concentration associated with dropped serum testosterone level were observed after oral administration of a wide range Al in male rats [4]. A recently published report on men (Sep. 2020) showed a reduction in spermatic motility and viability that positively correlated to high Al spermatozoal concentration [5].

The degenerative effect on the male reproductive tract may be attributed to alteration of redox balance, induction of free radicals cytotoxicity, and affection of enzymatic and biomolecules levels. The Al accumulation inhibits testosterone synthesis, induces testicular oxidative stress, interferes with gonadotrophin secretion by blockage of calcium voltage-gated channels leading to sperms necrosis and death [6].

*Echinacea purpurea* is one of the most important medicinally cultivated plants which was traditionally employed for the alleviation of many diseases. It has antiviral, larvicidal, antibacterial, and antioxidant activities. It is used for respiratory, urinary, cutaneous infections due to its immunostimulatory effect [7]. The plant contains many pharmacologically bioactive constituents that contribute to its biological activities, such as cichoricacid, alkamides, echinacoside, caffeic acid derivatives, chlorogenic acid, caftaric acid, β-sitosterol, polysaccharides, and glycoproteins [7].

The aim of our study was to evaluate the ability of *Echinacea purpurea* aqueous and ethanolic extracts to counteract the $AlCl_3$-induced male rat reproductive toxicity.

## Materials and Methods

### Rats

Sexually mature male Wistar rats weighing 150-170 g were used in this study. Rats were housed under hygieniclaboratory conditions with 12 hr. light/12 hr. dark cycle at temperature 23-25 °C. Animals were fed of rat pellets and water were provided *ad libitum* throughout the experiment period. All procedures were carried out according to the guidelines of the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo University (Approval number: Vet CU16072020176, dated 16 July, 2020).

### Herb Extraction and Chemicals

*Echinacea Purpura* flowers were obtained from the local market for Medicinal Plants and Herbs (Cairo, Egypt) and authenticated at the Herbarium of Botany Department, Faculty of Science, Cairo University. The herb flowers were air-dried at room temperature and pulverized using a metal grinder. Cold extraction was done by soaking 500 g of flower powder in 3 liters of 99% ethanol for 72 hr. at room temperature with intermittent shaking. The alcoholic extract was then concentrated in a rotatory evaporator at 50°C [8]. Each 500 g of pulverized flowers yielded 150 g semisolid extract. The extract was stored at 4°C until needed. For aqueous extract, the lyophilized powder was obtained from the Arab Company for Pharmaceuticals and Medicinal Plants, and the dose was adjusted by distilled water just before use. $AlCl_3$ and ethanol were obtained from Sigma-Aldrich Company in Egypt, Nasr City, Cairo.

### Preliminary Phytochemical Screening

Phytochemical screening for alkaloids, saponins, glycosides, resins, tannins and phenolic compounds was carried out according to the standard methods [9].

### Experimental Design

Rats were allocated into six groups of 11 animals in each. Group (1) was the negative control. Group (2) was given 150 mg/kg of *E. purpurea* aqueous extract only. Group (3) was given 150 mg/kg of *E. purpurea* ethanolic extract only. The given dose of the extract was adjusted to 150 mg/kg, as described [6]. Group (4) was given $AlCl_3$ in a dose of 100 mg/kg as a positive control as previously described [6]. Group (5) was given *E. purpurea* aqueous extract (150 mg/kg) concomitantly with $AlCl_3$ (100 mg/kg). Group (6) was given *E. purpurea* ethanolic extract concomitantly with $AlCl_3$ (100 mg/kg). Both $AlCl_3$ and *E. Purpurea* extracts were orally administered daily for 60 days. At the end of the study, all animals were euthanized under ether anesthesia. Blood samples were collected for

estimating serum testosterone levels. Testes were dissected out, and semen samples were collected from cauda epididymis for the examination of sperm characteristics. Histopathological examination of the testes was also performed.

**Epididymal Sperm Characters**

The epididymal contents of treated rats were obtained after cutting the tail of the epididymis, squeezing it gently on a clean slide. The progressive motility and sperm cell count were examined microscopically according to the method described [10].

**Testosterone Determination**

Testosterone levels were determined using the radioimmunoassay method (RIA) by a kit (catalog #1119) which is intended for the quantitative determination of total testosterone in the serum. This method is based upon the competitive binding principle. The unknown or standard samples were incubated with radioactive iodine125 (I125) labeled testosterone in antibody-coated tubes. After incubation, the liquid contents in the tubes were withdrawn and the bound radioactivity was determined using gamma counter according to the method described [9].

**Histopathological Examination of the Testes**

Testes of rats were dissected out after euthanasia and kept in 10 % neutral buffered formalin solution. The routine protocol of tissue specimen preparation was applied to obtain hematoxylin and eosin (H&E) stained sections [11]. Olympus (BX43) light microscope was used to examine the sections, and Olympus (DP-27) digital camera was used to capture images. The diameter of seminiferous tubules (µm) in each group was measured in the most circular tubules using ImageJ software (NIH, USA). Johnsen's score was used to evaluate the process of spermatogenesis by giving a score ranging from 1 to 10, describing the presence or absence of the cells of spermatogenesis series [12].

**Statistical Analysis**

Data are presented as mean ± SD and analyzed by a one-way ANOVA test using GraphPad (Prism).

**Results**

**Preliminary Phytochemical Screening**

Phytochemical screening of *E. purpurea* flower extract is recorded in Table 1. The results revealed presence of glycosides, tannins, phenolic compounds, and the absence of alkaloids, saponins, and resins.

**Table 1**: Phytochemical constituents of *E. Purpurea* ethanolic extract

| **Phytochemicals** | ***E. purpurea* ethanolic extract** |
|---|---|
| Alkaloids | - |
| Glycosides | ++ |
| Tannins | + |
| Phenolic compounds | +++ |
| Resins | - |
| Saponins | - |

(+ present; ++moderate percentage; +++ high percentage)

**Blood Testosterone Level**

Serum testosterone levels after various treatments were illustrated in (**Figure 1**).

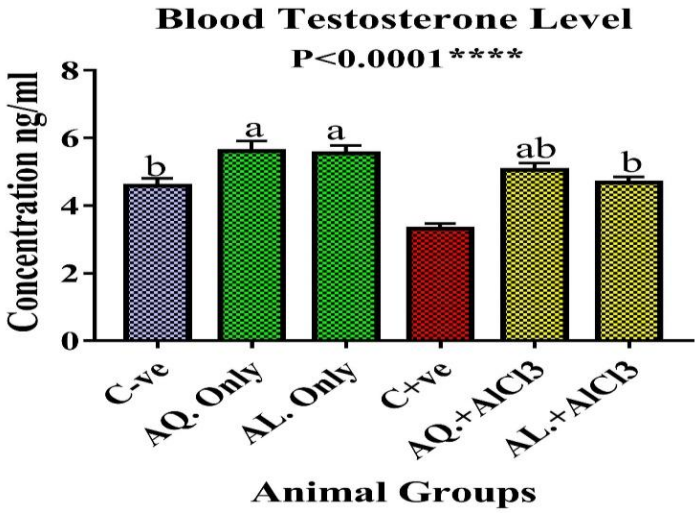


*Figure 1: Serum testosterone level*

**Figure 1.** Data are presented as means ± SD (n = 11); the means were compared by analysis of variance, post hock with Tukey; different letters or no letters indicate significant difference. Data analyzed by GraphPad (Prism). The groups were as follows: negative control (C-ve), *E. purpurea* aqueous extract 150 mg/kg (AQ. only), *E. purpurea* alcoholic extract 150 mg/kg (Al. only), positive control (C+ve), *E. purpurea* aqueous extract + $AlCl_3$ ($AQ+AlCl_3$), and *E. purpurea* ethanolic extract +$AlCl_3$ ($Al.+AlCl_3$).

**Semen Picture**

Sperm cell concentration, progressive motility percentage, live/dead ratio, and sperm abnormality were illustrated in (**Figure 2**).

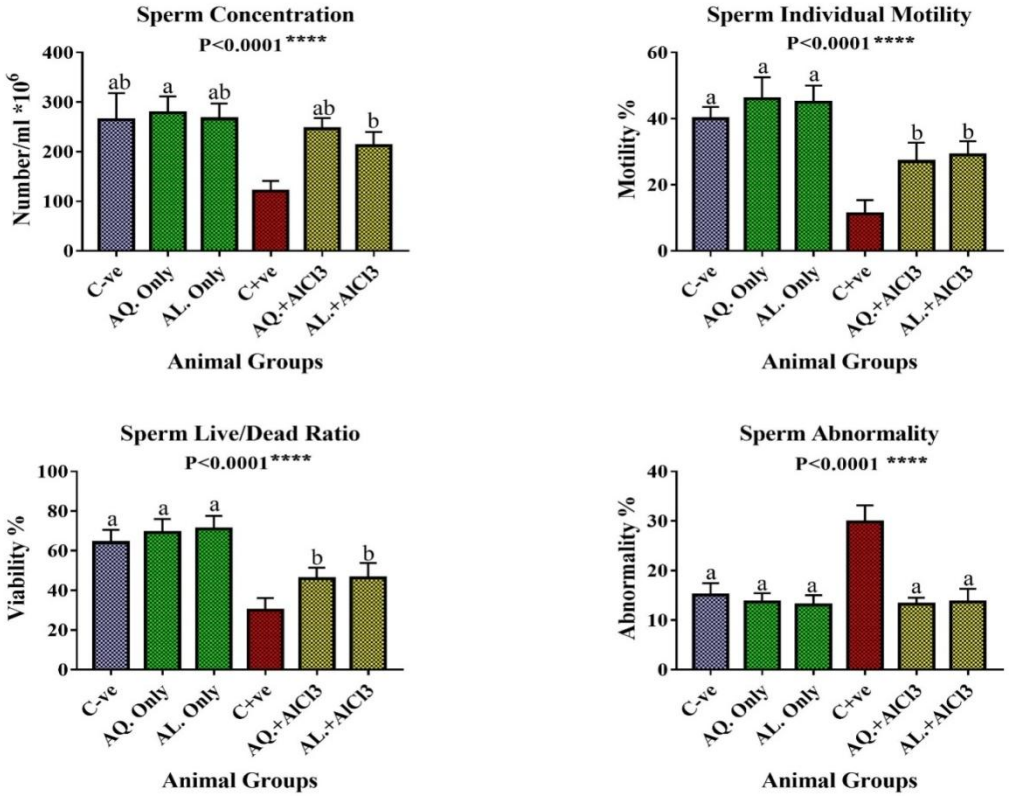


*Figure 2: Semen picture*

**Figure 2.** The data are presented as means ± SD (n = 11); the means were compared by analysis of variance, post hock with Tukey, different letters, or no letters indicate significant difference. Data analyzed by Graph Pad (Prism). The groups were as follows: negative control (C-ve), *E. purpurea* aqueous extract 150 mg/kg (AQ. only), *E. purpurea* alcoholic extract150 mg/kg (Al. only), positive control (C+ve), *E. purpurea* aqueous extract + $AlCl_3$ (AQ+$AlCl_3$), and *E. purpurea* ethanolic extract +$AlCl_3$ (Al.+$AlCl_3$).

**Histopathological Examination of the Testes**

Microscopic examination of the testes of rats of negative control group (**Figure 3**) revealed normal histology of the testes, in which the seminiferous tubules were formed of basement membrane resting on it the series of the spermatogenic cells and the lumen was filled with spermatozoa. Similarly, testes tissue from the groups received both *E. purpurea* aqueous and alcoholic extracts only (Figure 3b and c) appeared normal. The group received $AlCl_3$ (Figure 3d and f) showed various histopathological alterations; blood vessels congestion. Interstitial edema was frequently noticed. Testicular degeneration represented by decreased numbers of spermatogonial cells was observed in many sections. Some seminiferous tubules were nearly empty with thickened basement membrane and spermatid giant cell inside. Alcoholic extract treated group (Figure 3g and h) showed alleviated signs of $AlCl_3$ toxicity; few tubules suffered from mild degeneration and loss of spermatogonial cells. Likewise, the group treated with *E. purpurea* aqueous extract showed mild degenerating spermatocytes. The best protective action was achieved in the group receiving *E. purpurea* alcoholic extract; apparently, normal seminiferous tubules were observed in all examined sections.

The diameter of the seminiferous is illustrated in (**Figure 4**). No statistically significant difference was observed between the groups received either the *E. purpurea* aqueous or alcoholic extract alone and the negative control group. The model ($AlCl_3$) group showed a significant reduction in the diameter of the seminiferous group compared to the normal control group. Generally, both *E. purpurea* extracts were able to protect against $AlCl_3$, which is revealed by maintaining the diameter of the seminiferous tubules nearly as normal compared to the $AlCl_3$ group.

Regarding Johnsen's spermatogenesis score (**Figure 5**), the oral administration of $AlCl_3$ adversely affects the process of spermatogenesis; $AlCl_3$ group had a significant reduction in Johnsen's score compared to the other experimental groups. *E. purpurea* extracts were able to withstand $AlCl_3$ intoxication by maintaining a significantly higher spermatogenesis score compared to $AlCl_3$ group. The best protective effect was exerted by the *E. purpurea* alcoholic extract as demonstrated in (**Figures 4 and 5)**.

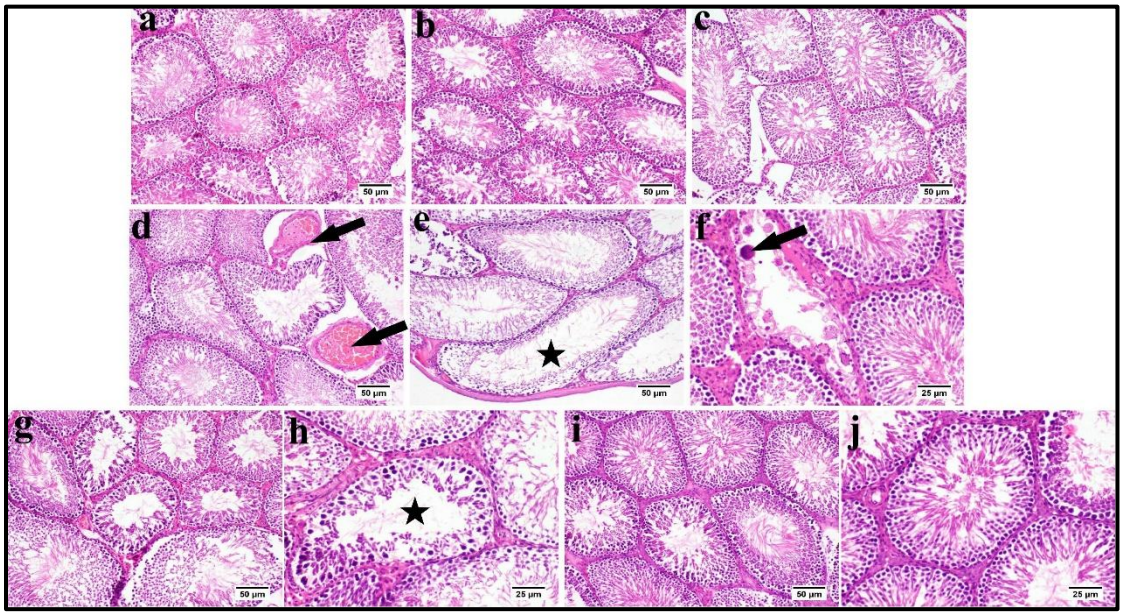


*Figure 3: Photomicrograph of the testes stained with H&E*

**Figure 3.** Photomicrograph of the testes stained with H&E: (a) Negative control group. (b) Aqueous extract only and (c) Alcoholic extract only, showing normal seminiferous tubules, (d) positive control ($AlCl_3$) group, showing congested blood vessels (arrows), (e) positive control ($AlCl_3$) group showing a marked decrease in spermatogenesis cells series (star), (f) positive control ($AlCl_3$) group, showing testicular degeneration with formation of spermatid giant cell (arrow) and (i) and (j) Aqueous extract+ AlCl3, showing mild reduction in spermatozoa and spermatids (star), (g) and (h) Alcoholic extract+ AlCl3, showing apparently normal seminiferous tubules.

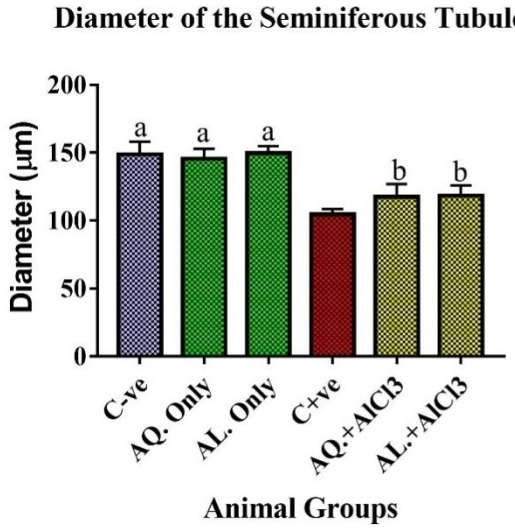


*Figure 4: Diameter (µm) of the seminiferous tubules in the different experimental groups and Johnsen's spermatogenesis score. Data were presented as means± SEM. a, b and c indicate significant difference at P<0.05.*

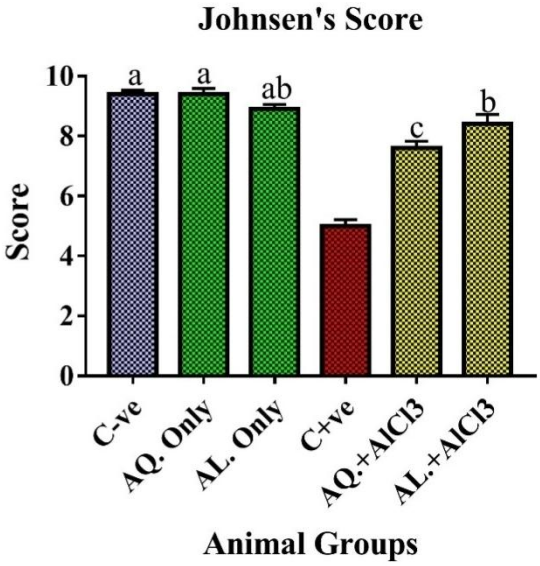


*Figure 5: Johnsen's spermatogenesis score. Data were presented as Data were presented as means± SEM. a, b, c, d and e indicate significant difference at P<0.05*

**Discussion**

Even Al ion has no biological role in the metabolic pathways, but it could lead to metallic toxicity to humans and animals due to exceeding the permissible exposure limit [13]. Continuous exposure to Al could induce damage to various systems, including skeletal, respiratory, nervous, hematopoietic system associated with increased oxidative stress [14].

Our results showed the negative deleterious effect of $AlCl_3$on the male reproductive system characterized by lower serum testosterone level, reduced sperm viability, increased sperm abnormalities, sluggish spermatic progressive motility associated with retarded spermatogenesis, and congested blood vessels. This is consistent with the previously reported studies concerned with the harmful effect of $AlCl_3$ on the male reproductive organs. $AlCl_3$is associated with male infertility, and reproductive disability [15] gonadal abnormalities [16] decrease testosterone levels in the testes and plasma of mice [17] abnormal rabbit sperm motility and viability [18]. The testicular accumulated Aluminum has been positively correlated with mice sperm necrosis [18].

Our study aimed to investigate the ability *E. purpurea* to withstand the deleterious effect of $AlCl_3$ on male reproductivity. The results showed that both *E. purpurea* aqueous and ethanolic extracts were significantly able to maintain the serum testosterone level, testicular structure integrity, and semen profile nearly to the normal healthy state. This comes in agreement with a previous study, reported the ability of *E. purpurea* extract to prevent testicular damage induced by testicular ischemia/reperfusion (I/R) injury in rats in a dose-dependent manner, which is reflected in the semen picture [19]. *E. purpurea* was also able to overcome the antiandrogenic effect of cyproterone acetate drug either prophylactically or through treatment via oral administration [20]. The protective effect of *E. purpurea* is attributed to its potent radical scavenging property, which is assigned to the presence of caffeic acid derivatives such as cichoric acid, lipophilic polyacetylene-derived compounds, such as alkylamides, echinacoside, and chlorogenic acid [19,20]. Also, the alkamides content of *E. purpurea* has a potent anti-inflammatory effect due to its ability to suppress COX-1 and to a lesser extent, COX-2 [21]. Moreover, *E. Purpurea* becomes one of the top-selling herbs due to its powerful antiaging effect, which is able to restore the aging markers to normal values of the young population, such as superoxide dismutase (SOD) and glutathione-s-transferase (GST), total cholesterol, HDL, LDL, VLDL, triglycerides, total protein, albumin, globulin, liver functions, hemoglobin concentration, WBCs, and platelets count [21].

In conclusion, *E. purpurea* extracts were able to overcome $AlCl_3$ induced- male reproductive toxicity by maintaining semen quality and quantity, increasing serum testosterone level, and ameliorate testicular histopathological damage and lesions. Therefore, this study recommends intake of *E. purpurea* herb flowers as a drink for patients suffering from sexual impotence; this is owing to its potent antioxidant and anti-inflammatory properties.

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