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EURYALE FEROX SALISB. AMELIORATES CADMIUM-INDUCED TESTICULAR AND HEPATIC IMPAIRMENTS IN MALE MOUSE MODEL

Ranjit Shaw[1,3], Shikhar Deep[1], Vidyanath Jha[2] and Radha Chaube[1]*

[1]Department of Zoology, Banaras Hindu University, Varanasi (U.P.), India.
[2]Department of Botany, Lalit Narayan Mithila University, Kameshwaranagar, Darbhanga (Bihar), India.
[3]Department of Biosciences and Bioengineering, IIT Bombay, Powai, Mumbai (M.S.), India

*Corresponding author: chauberadha@rediffmail.com

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Abstract: In humans, cadmium exhibits a potent compounding effect and is one of the major concerns, which can lead to several dangerous health hazards, including hostile reproductive health. There are several synthetic medications available to reduce its toxicity, but with a wide range of adverse effects. This scenario necessitates the pursuit of natural compound-based medicinal formulations. Therefore, the aim of the present study was to investigate the role of Makhana, *Euryale ferox* Salisb., against cadmium-induced testicular and hepatic pathophysiology, oxidative stress, hormone levels, and other parameters of testis and liver damage. In mice, its extract was administered orally on a daily basis for 21 days, and they were also subjected to Cadmium chloride solution using oral gavage. The animals were subjected to physical, biochemical, and histological analysis after the completion of dosage. Results indicated that Makhana extract has a significant therapeutic role in overcoming cadmium toxicity. The makhana ameliorates haematological indices, testis weight, hormonal levels, and histopathological alterations in a significant way. The makhana extract ameliorates cadmium-induced testicular and hepatic impairments, modulating testicular and hepatic histoarchitecture, steroid hormone, and oxidative stress. Thus, it can be suggested that foxnuts can be a potential candidate for treating cadmium toxicity.

Keywords: Anti-oxidant, Cadmium toxicity, *Euryale ferox*, Foxnut, Liver, Mouse, Testis, Toxicity.

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INTRODUCTION

Cadmium (Cd) is a dangerous, non-essential transition metal that can have adverse impact on animal and human health (Rahimzadeh *et al.*, 2017; Yasmeen, 2019; Kumar *et al.*, 2019). The Cd is one of the naturally occurring contaminants in the environment from industrial and agricultural sources (Kumar and Gupta, 2022). The heavy metals act as serious contaminants of the aquatic ecosystem, in view of the fact that they get accumulated by the aquatic creatures (Kaushal and Mishra, 2011; Verma and Prakash, 2019;

Prakash and Verma, 2020). Cadmium exposure mostly happens via consuming contaminated food or drink, although it can also be acquired by inhalation and smoking cigarettes. The industrial usage of cadmium as a corrosive reagent and as a stabilizer in PVC products, colour pigments, and Ni-Cd batteries are the consistent sources of cadmium contamination (Genchi *et al.,* 2020). Cadmium accumulates in plants and animals with a long half-life of about 25-30 years (Genchi *et al.,* 2020).

Human exposure to Cd can have several adverse consequences, including testicular damage (Tinkov *et al.,* 2018; Kumar and Sharma, 2019). The cadmium detoxification can be achieved with several synthetic compounds such as EDTA (ethylenediamine tetra acetic acid), BAL (British Anti-Lewisite, dimercaprol), DMSA (2,3-dimercapto-succinic acid), and DMPS (2,3-dimercapto-1-propane sulfonic acid) (Bernhoft, 2013). However, these synthetic compounds come with various side effects on the body. Thus, several naturally occurring biologically active substances found in plants may help to reduce the harmful health effects of exposure to heavy metals, such as cadmium.

In routine life, humans come into contact with heavy metals, which can have harmful effects on the male reproductive system, including spermatogenesis, semen quality, and the synthesis and release of reproductive hormones. It has been observed that cadmium can impact testicular function, hormonal balance, and semen quality indices in several animal species (Oliveira *et al.,* 2009; Alaee *et al.,* 2014; Zhao *et al.,* 2015; Wang *et al.,* 2017; Boujelben *et al.,* 2018).

After a review of experimental studies on the effects of cadmium on reproduction, it was determined that the induction of subfertility following the administration of cadmium may have been caused by the metal penetrating testicular tissue and causing damage to the tissue, which would have impaired testicular function. This is evidenced by the disruption of spermatogenesis and sperm motility, either with or without affecting the male reproductive endocrine function (Alaee *et al.,* 2014). The aquatic crop *Euryale ferox* sometimes referred to as 'makhana or foxnut,' belongs to the Nymphaeaceae family (Devi *et al.,* 2020). It is mostly sold in South and East Asian nations, including India, China, Nepal, Bangladesh, Japan, Russia, Korea, and others. Its cultivation in India is restricted to a few states, including Bihar, Assam, Manipur, West Odisha, Tripura, and Bengal (Kumar and Gupta, 2022). *Euryale ferox* Salisb., the only species in the genus *Euryale* of the family, Nymphaeaceae, is a well-known edible and medicinal aquatic plant (Rathod *et al.,* 2023). The young stalks and rhizomes of this plant are edible, and the seeds, known as Qianshi as well as cock's head in Chinese, are consumed medicinally or as food (Song *et al.,* 2011).

In Chinese and Ayurvedic medicine, makhana seeds are widely used and have been shown to have therapeutic benefits for treating a variety of ailments, such as renal disease, persistent diarrhoea, severe leucorrhoea, and splenic hypofunction (Kumari e*t al.,* 2019). Makhana has recently gained international notice because of its nutritional and therapeutic properties (Kumari and Jha, 2017). Its potent therapeutic qualities against various human illnesses affecting the respiratory, circulatory, digestive, excretory, and reproductive systems are indicated by references from ancient India and China (Jha *et al.,* 1991).

Makhana is considered a super-food because of its high nutritional content. It has numerous vital functions in the human body, some of which include aids in preventing aging, helps in improving cognitive functions, in treating infertility and erectile dysfunction, etc. In women, it assists in strengthening the uterine lining; in males, it helps boost testosterone levels. It is an ideal snack for diabetic patients because of its low glycemic index.

Biological studies indicated that *E. ferox* possesses antioxidant, anti-aging, anti-fatigue, antidepressant, and anti-diabetic properties, among many other medicinal values (Song *et al.,* 2011; Wu *et al.,* 2014; Ahmed *et al.,* 2015; Tehseen *et al.,* 2020). The shell, petiole, pedicel, and seeds of the plant have all recently been researched for their potency in diabetes treatment (Rathod *et al.,*

2023). It was reported that the triterpenoid-rich extracts from the shell could regulate glucose metabolism in diabetes-induced mice by alleviating insulin resistance (Yuan *et al.,* 2013). In addition, the alcoholic extract from seeds could improve glycemic control and lipid profile in diabetic rats, along with antioxidant enzyme levels (Ahmed *et al.,* 2015). Despite the above-mentioned pharmacological potential, no activity has been explored against Cd-induced toxicity. Thus, the current work aims to explore the anti-toxicity potential of *Euryale ferox* Salisb. popped seed extract in mice model exposed to cadmium chloride.

MATERIALS AND METHODS

1. Chemicals and Reagents

All chemicals used in the study were of analytical grade and were used without purification. Milli-Q system (Millipore Corp., Bedford, MA) was used for double distillation and deionization of water.

2. Animal Work

The study has been conducted in the Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India. Healthy male BALB/c strain mice, 70-90 days old, weighing ~25-30 g, were used for the experiments. All animals were maintained in-house under standard conditions, viz., 50% humidity, and a temperature of 22 ± 2°C having a 12 h dark/light cycle. Food and water were supplied *ad libitum*. The animals were maintained according to the guidelines of the Committee for the Purpose of Control and Supervision for Experiments on Animals, Government of India (CPCSEA). At the end of the study, mice were decapitated.

3. Experimental design

20 Male BALB/c strain mice were used to evaluate the impact of foxnut on Cadmium chloride-treated mice *in vivo*. For this, mice were randomly divided into four groups (5 mice/group). Initially, the mice were acclimatized for 6-7 days and then were divided into 4 groups evenly and subjected to:

(1) administration of vehicle by oral gavage, which served as Control (CN) group;

(2) administration of Cadmium chloride (CC) (6 mg/kg body weight) by oral gavage;

(3) administration of only ethanolic foxnut extract (FE)(20 mg/kg body weight) by oral gavage;

(4) administration of both ethanolic Foxnut extract (20 mg/kg body weight) as well as Cadmium Chloride (6 mg/kg body weight) (CC + FE) by oral gavage.

Cadmium chloride was dissolved in water. Popped foxnut seeds were pulverized and then dissolved in water and ethanol. The experiment was conducted for 21 days.

4. Collection of Tissue and Blood

After dose administration for 21 days, the mice were sacrificed by decapitation to collect blood. Serum was isolated from the blood samples by centrifuging whole blood at 1000×g for 10 minutes, and it was then kept for further analysis at −20 °C. The tissues, such as the liver and testis, were harvested, weighed, and fixed in Bouin's fixative for histological studies, and a part of the tissues were stored at -20^{0}C for further biochemical analysis.

5. Determination of Sperm motility

The motility of sperm was determined in control and experimental sets. For this, the mice's epididymis was first harvested and dilacerated in 1 mL of M2 medium (Sigma-Aldrich, MO, USA), and spermatozoa were allowed to swim out for 10 min at 37 °C. The motility of the spermatozoa was evaluated with an Olympus microscope and Computer Aided Sperm Analysis (CASA) (CEROS II apparatus; Hamilton Thorne).

6. Determination of Oxidative Stress Parameters

To determine the effect of foxnut extract on oxidative stress induced in the experimental sets, Catalase and Superoxide Dismutase (SOD) assays were performed. At first, 10% homogenate of the tissues (liver and testis) was prepared, for which 10 mg of tissue was weighed and homogenized in 1 ml of PBS, pH 7.4. This was followed by centrifugation at 4^{0}C at 12,000×g, after which the pellet was discarded, and the supernatant was collected and stored at -20^{0}C for further studies.

Protein estimation by Bradford assay:

The amount of protein in the tissue homogenate was determined using the Bradford assay. For

this, the standard curve was prepared using more than 5 known concentrations of Bovine Serum Albumin (BSA), which was employed to calculate the amount of total protein present in the sample. Thus, 5 uL of each of the five concentrations of BSA and 5 uL of 20 times diluted homogenate was loaded into the wells of a 96-well plate (Costar, Corning, NY, USA), followed by the addition of 200 uL Bradford reagent (Hi Media, India) in each well. The absorbance at 580nm was measured using a microplate reader (Molecular Devices, USA).

Catalase assay:

The level of Catalase enzyme in the testis and liver was estimated following the standard protocol by Sinha (1972). To perform this assay, 500μl of H_2O_2 was added to the glass test tubes, followed by the addition of 625uL of PBS, pH 7.4, and 125uL of 10 % tissue homogenate to each tube. After 20 seconds, 2mL of the reaction mixture (Potassium dichromate and Glacial acetic acid in a ratio of 1:3) was added to each tube and incubated in the water bath at 100^{0}C for 10 minutes. Then, the test tubes were allowed to cool down to room temperature, after which 250uL of solution from each tube was taken and loaded onto a 96-well plate (Costar, Corning, NY, USA). The absorbance at 570nm was measured using a microplate reader (Molecular Devices, USA).

Superoxide dismutase (SOD) assay:

The level of SOD in the testis and liver was determined following the method postulated by Campos-Shimada *et al*. (2020). Superoxide protects the cells by dismutating superoxide radicals into hydrogen peroxide and water. Here, the superoxide radical generation was achieved by photoreduction of Riboflavin and combined with nitrite formation from Hydroxylamine Hydrochloride. The superoxide radical reacts with nitrite, which then reacts with Sulphanilic acid to produce a diazonium compound. This reacts with the Griess reagent to give a red azo compound whose absorbance is measured at 543nm.

To execute this assay, 1.4 ml of reaction mixture was added into each glass test tube, followed by the addition of 100 uL of tissue homogenate. The tubes were subjected to a brief incubation at 37^{0}C for 10 minutes. Then, Riboflavin was added to each tube, and the tubes were exposed for 10 minutes in a 20 W Philips fluorescent bulb. Next, 1 mL of Griess Reagent was added to each test tube. At last, a fixed volume (250uL) was taken from each tube and loaded onto a 96-well plate (Costar, Corning, NY, USA). The absorbance at 543 nm was measured using a microplate reader (Molecular Devices, USA).

7. Profiling of hormone parameters

The levels of two sex hormones, *i.e.,* oestradiol and testosterone, were estimated using a Competitive enzyme-linked immunosorbent assay (ELISA). ELISA for estradiol and testosterone was performed using Diametra Estradiol and DRG Testosterone ELISA kit as per the manufacturer's instructions, respectively. The Serum samples and reagents were brought to room temperature before use. 25ul of each calibrator and serum were loaded onto the wells of a 96-well plate (Costar, Corning, NY, USA), followed by the addition of 200uL of the estradiol-HRP conjugate. Testosterone-HRP conjugate was used in the case of testosterone ELISA. The well plate was incubated at 37^{0}C for 1 hour. After incubation, the contents were removed, and the wells were washed three times with 300uL of 10X wash buffer in order to remove any unbound antigens. Following this, 200μL of TMB substrate was added to each well and incubated for 15 minutes at room temperature. In the end, a 100 uL stop solution was added and gently shaken. Absorbance was measured at 450nm within 10 minutes of adding stop solution in a microplate reader (Molecular Devices, USA). The intensity of color developed is inversely proportional to the concentration of estradiol or testosterone in the sample.

8. RNA isolation and quantitative real-time PCR (qRT-PCR)

To investigate the expression profile of the *Cyp19A1* (aromatase) gene in the testicular cells of all the groups, RNA was isolated from the testis, followed by cDNA synthesis. Gene expression was analyzed by quantitative real-time PCR using the protocol by Chaube *et al*. (2015). Total RNA was extracted from testis tissues stored in RNA later

using the RNeasy mini kit, according to the manufacturer's protocol. Total RNA (5 μg) was reverse transcribed using random hexamer primers and Revert Aid M-MuL V reverse transcriptase in a 20 μL reaction volume (first strand cDNA synthesis kit, Fermentas) using the manufacturer's protocol. Gene-specific primers for mice aromatase gene and β-actin were used in the PCR (Table 1). Quantitative PCR assays were performed in triplicate for different samples using forward and reverse primers and VeriQuest TM SYBR Green qPCR master mix with ROX (Afymetrix, Inc. Cleveland, Ohio USA) in an ABI Prism 7500 thermal cycler (Applied Biosystems, Foster, CA, USA) at 95 ℃ (15 s) and 60 ℃ (1 min) for 40 cycles. Each sample was run in a final volume of 20 μL containing 1 μL of cDNA, 10 pM of specific primer, and 10 μL of SYBR Green PCR master mix. The specificity of amplicons was verified by melting curve analysis (60–95 ℃) after 40 PCR cycles. As controls, the assays were performed without templates and reverse transcriptase. No amplification was observed in the control samples. Cycle threshold (Ct) values were obtained from the exponential phase of PCR amplification, and the target gene expression was normalized against mice β-actin expression to generate $2^{-\Delta\Delta Ct}$ values to quantify the target gene abundance (Livak and Schmittgen, 2001).

Table 1: List of primers used in real-time qPCR for gene expression study.

Serial No.	Name of gene	Sequence of primers
1	Cyp19A1	Forward: 5'-ATCCACTGGCGGGTTTTCTCTAT-3' Reverse: 3'-CTTGGTCCCGATTCCCATCTACG-5'
2	β-actin	Forward: 5'-CCATACAGTGTGGGTGAGTCTT-3' Reverse: 3'-AGGTCGGCCACCTTCCGTCA-5'

9. Histological analysis

On the last day of treatment in all the groups, the organs of the mice, such as the liver and the testis, were collected and fixed overnight in 10% formalin (Merck, Germany), dehydrated, embedded, sectioned, and then stained using Haematoxylin and Eosin, so as to assess the gross histopathological changes or, lesions in the respective organs. The slides were imaged in a bright field using a Nikon Eclipse Ti2 inverted microscope fitted with a Color Camera Nikon DS-Ri2 at 40X magnification.

10. Statistical analysis

The statistical data were presented as mean ± SD with one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test, employing the GraphPad Prism 9.5.1 (GraphPad Software, San Diego, CA, USA). A p-value less than 0.05 were considered as indicative of significance. The statistical significance was calculated using ANOVA (95% confidence interval) with p values, $p < 0.0001$, $p < 0.0002$, $p < 0.0021$, $p < 0.0332$, and $p > 0.1234$ indicated by (****), (***), (**), (*) and (ns), respectively.

RESULTS AND DISCUSSION

Because of modern technology and industries' rapid expansion, cadmium has been introduced into the environment as a contaminant (Sirot *et al.,* 2008). It gets absorbed in abundant quantities via water, food, and air contaminations. Changes in steroidogenesis, irregularities in the menstrual cycle and reproductive hormones, delayed puberty and menarche, loss of pregnancy, preterm birth, and low birth weight are all brought on by cadmium (Thompson and Bannigan, 2008).

In Asia, the plant *Euryale ferox* Salisb. is referred to as gorgon nut and Phool makhana. Given the abundance of macronutrients, micronutrients, and amino acids, it has nutritional value and is easy to consume in various recipes, such as snacks, rice pudding, and desserts (Nehal *et al.,* 2015). Makhana seeds have also been shown to have medicinal qualities. They are frequently used in Ayurvedic and Chinese medicinal formulations to treat a range of wide arena of illnesses, including renal failure, diarrhoea,

excessive leucorrhoea, and hypofunction of the spleen (Kumari *et al.,* 2019).

Several investigational approaches proved that Makhana seeds exhibit a plethora of various pharmacological activities, including anti-diabetic, anti-fatigue, anti-spermatorrhoea, ulcer protective, anti-cancer, anti-oxidant, anti-diabetic nephropathy (Song *et al.,* 2011; Jha *et al.,* 2014; Zhang *et al.,* 2019; Mittal *et al.,* 2020), anti-hyperlipidaemic, hepato-protective (Yuan et *al.,* 2013; Ahmed *et al.,* 2015), anti-melanogenic (Baek *et al.,* 2015), anti-arthritic, cardio-protective (Das *et al.,* 2006), and gastro-retentive (Negi *et al.,* 2011). According to Zhao *et al.* (1989), the active ingredient in this plant, glucosyl sterols, is the one that has bestowed this nut with its therapeutic qualities. Plant products' antioxidant activity has been investigated in relation to their medicinal qualities, which include a decrease in ischemia/reperfusion heart injury (Das *et al.,* 2006; Lee *et al.,* 2002). According to a recent Chinese study, makhana's petioles and pedicels have strong antioxidant properties (Wu *et al.,* 2014). Numerous additional worldwide researches have highlighted this plant's numerous therapeutic uses (Guo *et al.,* 1993; Song and Wu, 2009; Zhang, 2009).

In the current work, a mouse model of cadmium-induced testicular dysfunction was used to examine the impact of foxnuts. According to our findings, foxnut extract has a protective effect on the study's parameters. As demonstrated in Fig. 1A, the testis weight of the mice decreased significantly due to the toxicity posed by cadmium chloride. However, the animals were able to maintain their normal testis weight when subjected to foxnut extracts along with cadmium chloride, which is comparable to the vehicle control. As shown in Fig. 1B, CC Group animals demonstrated a significant loss in sperm motility when compared to the CN set of animals. This sperm parameter was recovered in the CC + FE set of animals compared to the CC group.

Thus, the cadmium chloride-treated animals had decreased sperm motility and testis weight; however, ethanolic extract of foxnut at a dose of 20 mg/kg significantly enhanced these parameters.

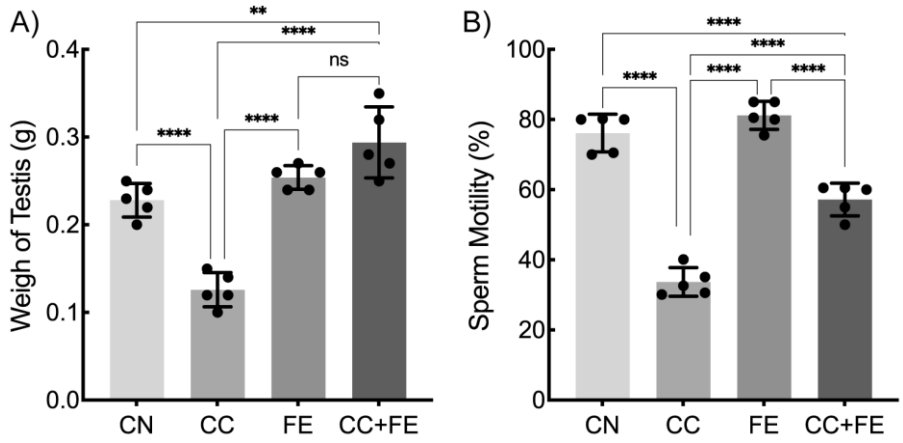

Fig.1: Effect of Foxnut extracts on the weight of the testis (A) and Sperm motility (%) (B) of animals subjected to cadmium toxicity, respectively.

Note: For fig. 1 to 5, the data represents the mean ± SD (n=5). The statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison post hoc analysis, with a 95% confidence interval. $p < 0.0001$, $p < 0.0002$, $p < 0.0021$, $p < 0.0332$, and $p > 0.1234$ is indicated by (****), (***), (**), (*), and (ns) respectively.

Increased oxidative stress and decreased antioxidant defense systems have also been linked to cadmium-mediated testicular dysfunction and reduced fertility (Liu *et al.,* 2009; Patra *et al.,* 2011). Foxnut extracts are well-known to possess increased rates of radical scavenging properties when tested with DPPH (1,1-diphenyl-2-picrylhydrazyl), TEAC (Trolox Equivalent Antioxidant Capacity), and CAT, SOD activity, and thus termed as an antioxidant, preventing lipid peroxidation (Tehseen *et al.,* 2020).

The total antioxidant potential of the foxnut extracts was analyzed by estimating the levels of SOD and Catalase in the liver and testis tissue samples. As is evident from Fig. 2, foxnut extracts showed protective effects against cadmium-induced oxidative damage in mice testis. The levels of Catalase, as well as SOD, decreased significantly in Cadmium-treated groups, which is a crystal clear indication of oxidative stress in the animals. However, the stress levels decreased in the third and fourth groups when they were subjected to makhana treatment. The oxidative stress level was also assessed in the liver of mice, which also showed a similar pattern of observation as in the case of testis in the treatment groups (Fig. 3).

Therefore, cadmium toxicity was found to cause oxidative stress in the testis as well as in the liver, and a dose of foxnut extract significantly reduced these levels. The levels of both enzymes appeared to be recovered by treating the cadmium-exposed male mice with the foxnut extract. The potential therapeutic benefit of makhana seeds in reducing the effects of cadmium toxicity may be attributed to their antioxidant-active constituents.

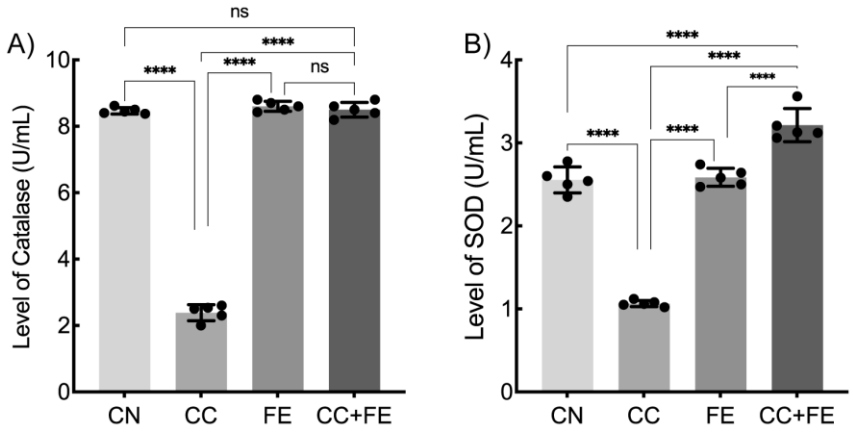

Fig. 2: Determination of effects of foxnut extract on the oxidative stress of treatment animal groups in the testis.

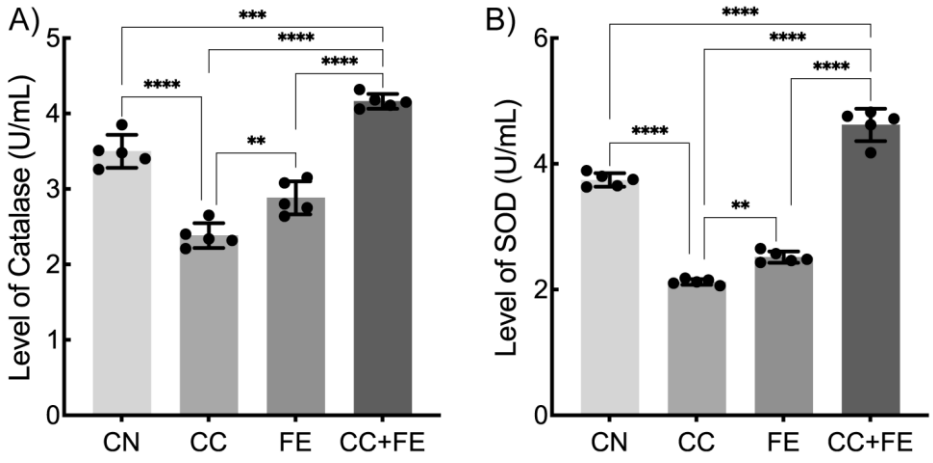

Fig.3: Determination of effects of foxnut extract on the oxidative stress of treatment animal groups in the liver.

Testis function is also significantly regulated by testosterone (Jeremy *et al.,* 2022). Therefore, the titer of testosterone hormone in circulation was determined. The data revealed that in comparison to the CN group, the circulating testosterone in the CC groups significantly dropped (Fig. 4A). When compared to the CC group, the administration of foxnut extract at a dose of 20 mg/kg along with cadmium chloride markedly increased the level of circulating testosterone. Thus, as per findings, cadmium toxicity reduced the levels of testosterone in the

blood; nonetheless, only a small amount of foxnut extracts (20 mg/kg) significantly raised the levels of testosterone in the blood.

It has been demonstrated that spermatogenesis and testicular proliferation depend on oestrogen and oestrogen receptors (Kucukler *et al.,* 2020). Estradiol (17β-Estradiol) represents the major estrogen in humans. Because cadmium binds to estrogen receptors and activates extracellular signal-regulated kinases 1 and 2, it can raise estradiol levels in humans. The result showed that the level of Estradiol-17 β increased significantly when treated with cadmium, which got little normalized in the animal group that was treated with both cadmium and foxnut extract (Fig. 4B). Thus, the findings demonstrate that the circulating levels of estradiol are increased due to cadmium toxicity; however, 20 mg/kg of ethanol foxnut extract slightly normalized its levels.

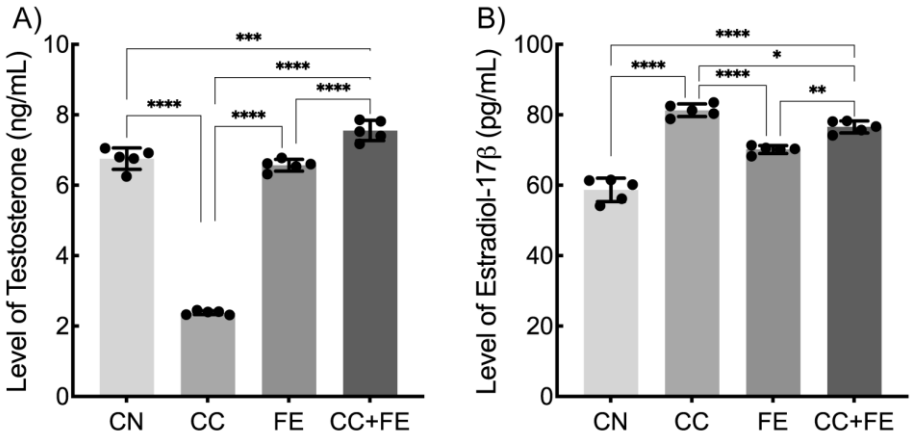

Fig. 4: Effect of Foxnut extracts on Testosterone (A) and Estradiol-17 β (B) levels of the animals subjected to cadmium toxicity.

One of the crucial enzymes responsible for estrogen biosynthesis is aromatase, also known as estrogen synthase or Cyp19A1. They catalyze several reactions which are involved in the pathway of steroidogenesis. The process via which androgens are aromatized into estrogens is specifically carried out by aromatase. Thus, the mRNA level of this gene was determined too. As seen in Fig. 5, the mRNA level of the aromatase/*Cyp19A1* gene significantly dropped in the cadmium-treated group of animals; however, the level was restored when subjected to makhana treatment daily. Thus, it was seen that the level of the *Cyp19A1* gene diminished in cadmium-treated mice, while it was restored in mice exposed to makhana treatment for 21 days.

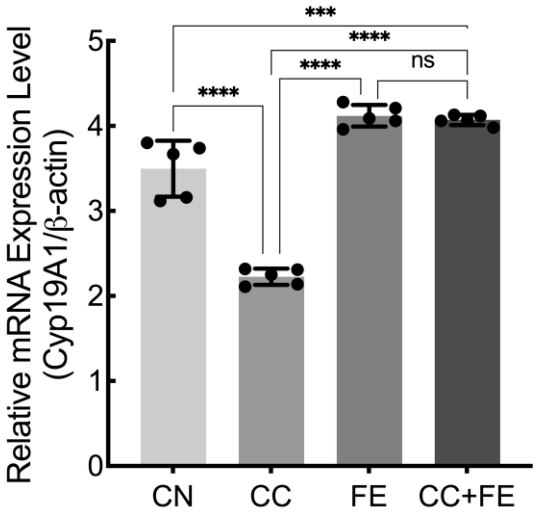

Fig. 5: Determination of relative mRNA level of aromatase/Cyp19A1 gene in the testis of treated animal groups.

Authors also evaluated the histopathological changes induced by cadmium and the foxnut extract. Fig. 6 displays the results of H and E staining for histopathological assessment. There is a distinct abnormality in the morphology of testis and liver cells in the cadmium-treated groups, which disappeared in the fourth group and resembled that of the control group.

In the present study, compared to control sections, $CdCl_2$-treated testis tissue sections showed seminiferous epithelium degeneration, death of germ cells, increase in interstitial cells and space, and Leydig cell damages. FE showed intact seminiferous epithelium with a high proliferation of spermatogonia cells. FE+$CdCl_2$ showed degenerated seminiferous tubules with vacuolization, reduced interstitial cells, and vacuolization.

Authors observed the histopathological changes in the mice liver exposed to cadmium, *Euryale ferox* and *Euryale ferox* + $CdCl_2$ treatments. The liver sections exposed to cadmium showed generalized degeneration, damage to hepatic cords, depletion of hepatocytes, depletion of hepatocyte parenchyma sheath, necrosis, and vacuolization. $CdCl_2$-induced degenerative changes were evident in numerous hepatocytes. Sections treated with *E. ferox* showed a central vein, hepatic artery, and binucleated hepatocyte. FE + $CdCl_2$ showed enlarged hepatocytes with an increase in the number of uninucleate hepatocytes and exhibited hepatic cords, nuclear hypertrophy, and vacuolization.

Thus, in terms of histopathological changes, the morphology of cells in the liver and testis became abnormal due to cadmium toxicity, which changed back to normal morphology after the treatment with foxnut extract.

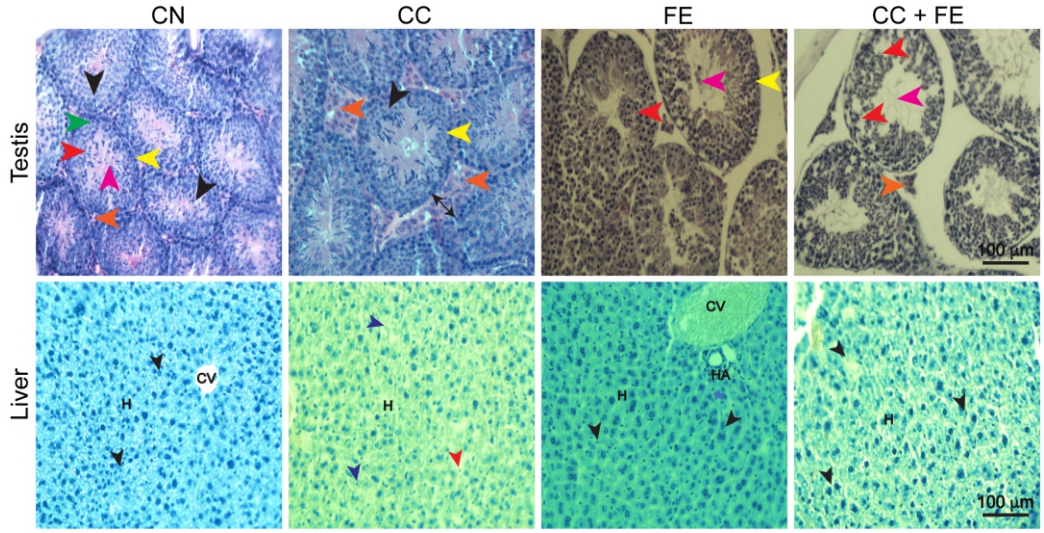

Fig. 6: Representative photographs of histological morphology of testis and liver stained with H and E (20X magnification, the scale bar is 100 µm).

In the upper panel of testis, Black arrow: Seminiferous tubules with spermatogenic cells; Yellow arrow: Spermatogonium; Green arrow: Spermatocyte; Red arrow: Spermatids; Pink arrow: Spermatozoa; Orange arrow: Leydig cells; Black double sided arrow: Interstitial Space. In the lower panel of the liver, Black arrow: Hepatic cords; Blue arrow: Generalised Degeneration; Red arrow: Necrosis; H: Hepatocytes; HA: Hepatic artery; CV: Central Vein.

CONCLUSIONS

One of the most hazardous substances to which people may be exposed at work or in the environment is cadmium, which is harmful to the male reproductive system as well. *Euryale ferox* has a potential therapeutic effect to rescue against cadmium-induced toxicity in the presented experimental work. The extracts of this medicinal plant are implicated in increasing cell motility and augmenting the performance of several antioxidant enzymes. Foxnut extracts are seen to increase sperm cell motility. The possible effects on cadmium toxicity could probably be interrelated with the existence of antioxidant-

active components present in the extract. Hence, this study supports the pharmacological facts regarding the management of manifestations related to cadmium toxicity.

CONFLICT OF INTERESTS

The author(s) declare no financial competing interest.

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RC and RS conceived the project and planned the work. RS and SD conducted and carried out the experiments and acquired the data unless mentioned otherwise. RS wrote and prepared the manuscript as well as formatted all the figures and graphs. VJ and RC helped in analysing and reviewing the manuscript. All authors read and approved the final manuscript. The authors acknowledge the Central facility of the Department of Zoology, Institute of Science, BHU for their Leica Microscopy facility. The authors also acknowledge Dr. Richa Arya for allowing them to use their microscopy facility. This work was supported by the "Department of Science and Technology (DST) Project" (Grant Id: P-1302) and Departmental funds. Besides, the authors thank Rabsang Lhamo and Raunak Kumar, for assisting in the experiments.

REFERENCES

1. **Ahmed D., Sharma M., Kumar V., Bajaj H.K. and Verma A.** (2015). 2β-hydroxybetulinic acid 3β-caprylate: an active principle from *Euryale ferox* Salisb. seeds with antidiabetic, antioxidant, pancreas and hepatoprotective potential in streptozotocin induced diabetic rats. *Journal of Food Science and Technology*. 52: 5427-5441.
2. **Alaee S., Talaiekhozani A., Rezaee S., Alaee K. and Yousefian E.** (2014). Cadmium and male infertility. *Journal of Infertility and Reproductive Biology*. 2(2):62-69. https://www.dormaj.org/index.php/JIRB/article/view/376
3. **Baek S.H., Nam I.J., Kwak H.S., Kim K.C. and Lee S.H.** (2015). Cellular anti-melanogenic effects of a *Euryale ferox* seed extract ethyl acetate fraction via the lysosomal degradation machinery. *International Journal of Molecular Sciences*. 16(5):9217-9235. https://doi.org/10.3390/ijms16059217
4. **Bernhoft R.A.** (2013). Cadmium toxicity and treatment. *The Scientific World Journal*. 1: 394652. https://doi.org/10.1155/2013/394652
5. **Boujelben M., Abdennabi R., Guermazi F. and Elfeki A.** (2018). Impact of cadmium on the endocrine and exocrine sexual activity in the adult male and female wistar rats: Determination of an apoptotic process. *Journal of Environmental & Analytical Toxicology*. 8:2161-0525. https://doi.org/10.4172/2161-0525.1000552
6. **Campos-Shimada L.B., Hideo Gilglioni E. *et al.*** (2020). Superoxide dismutase: a review and a modified protocol for activities measurements in rat livers. *Archives of Physiology and Biochemistry*. 126(4):292-299.
7. **Chaube R., Joy K.P. and Acharjee A.** (2015). Catfish gonadotrophins: cellular origin, structural properties and physiology. *Journal of Neuroendocrinology*. 27(6):536-543.
8. **Das S., Der P., Raychaudhuri U., Maulik N. and Das D.K.** (2006). The effect of Euryale ferox (Makhana), an herb of aquatic origin, on myocardial ischemic reperfusion injury. *Molecular and Cellular Biochemistry*. 289: 55-63. 10.1007/s11010-006-9147-1
9. **Devi M., Sharma K., Narayan J.S., Arora S., Patel S., Kumar Y. and Kumar V. R.** (2020). Effect of popping on physicochemical, technological, antioxidant, and microstructural properties of makhana seed. *Journal of Food Processing and Preservation*. 44(10):e14787.https://doi.org/ 10.1111/jfpp.14787
10. **Genchi G., Carocci A., Lauria G., Sinicropi M.S. and Catalano A.** (2020). Nickel: Human health and environmental toxicology. *International Journal of Environmental Research and Public Health*. 17(3): 679. https://doi.org/10.3390/ijerph17030679
11. **Guo R.A., Tang X.H., Sun D.J., Li Z.F. and Liu C.Y.** (1993). Case study of thirty-seven patients with proteinuria inhibitor by Qianshi. *Journal of Shangdong College of Traditional Chinese Medicine*.17:32-33.
12. **Jeremy M., Kharwar R.K. and Roy V.K.** (2022). Synthetic leptin c-fragment peptide

minimises heat-induced impairment of spermatogenesis in mice via Stat3signalling. *Theriogenology*. 178:40-49. https://doi.org/10.1016/j.theriogenology.2021.10.028

13. **Jha V., Kargupta A.N., Dutta R.N., Jha U.N., Mishra R.K. and Saraswati K.C.** (1991). Utilization and conservation of *Euryale ferox* Salisbury in Mathila (North Bihar), India. *Aquatic Botany*. 39(3-4):295-314. https://doi.org/10.1016/0304-3770(91)90005-P
14. **Jha V., Verma A.B. and Jha P.** (2014). Aquatic macrophytes effective in control of diabetes: A review. *Annals of Plant Sciences*. 3(3): 645-650.
15. **Kaushal B.T and Mishra A.** (2011). A comparative toxicity analysis of cadmium compounds on morphological and behavioral aspects in air breathing freshwater fish *Channa punctatus*. *International Journal of Science and Nature*. 2:266-269.
16. **Kucukler S., Caglayan C., Darendelioglu E. and Kandemir F.M.** (2020). Morin attenuates acrylamide-induced testicular toxicity in rats by regulating the NF- B, Bax/Bcl-2 and PI3K/Akt/mTOR signaling pathways. *Life Sciences*. 261: 118301. https://doi.org/10.1016/j.lfs.2020.118301
17. **Kumar A., Prakash S. Parmar A. and Bajpeyee A.K.** (2019). Effect of cadmium on fresh water teleost, *Heteropneustes fossilis* (Bloch). *International Journal of Biological Innovations*. 1 (1):14-17. https://doi.org/10.46505/IJBI.2019.1103
18. **Kumar S. and Gupta P.** (2022). Impact of over cultivation of Makhana, *Euryale ferox* Salisb. *International Journal of Biological Innovations*. 4(2): 313-321. https://doi.org/10.46505/IJBI.2022.4208.
19. **Kumar S. and Sharma A.** (2019). Cadmium toxicity: effects on human reproduction and fertility. *Reviews on Environmental Health*. 34(4):327-338. https://doi.org/10.1515/reveh-2019-0016
20. **Kumari A. and Jha V.** (2017). Ethnic uses of Makhana (*Euryale ferox* Salisb.) in Mithila (north Bihar) and other parts of India. *Journal of Traditional and Folk Practices*. 5(1). https://doi.org/10.25173/jtfp.2017.5.1.65
21. **Kumari R., Jakhar D.S. and Kumar P.** (2019). Nutritional and medicinal importance of makhana (*Euryale ferox* Salisb.). *Marumegh*. 4:53-55.
22. **Lee S.E., Ju E.M. and Kim J.H.** (2002). Antioxidant activity of extracts from Euryale ferox seed. *Experimental & Molecular Medicine*. 34(2):100-106.
23. **Liu J., Qu W. and Kadiiska M.B.** (2009). Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicology and Applied Pharmacology*. 238(3):209-214. https://doi.org/10.1016/j.taap.2009.01.029
24. **Livak K.J. and Schmittgen T.D.** (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*. 25(4): 402-408. https://doi.org/10.1006/meth.2001.1262
25. **Mittal R., Sharma S. and Mittal A.** (2020). A Critical Review on Ethnobotanical and Pharmacological Aspects of *Euryale ferox* Salisb. *Pharmacognosy Journal*. 12(6): 10.5530/pj.2020.12.199
26. **Negi J.S., Jugran V. and Kasliwal N.** (2011). Development of non-effervescent floating matrix tablets based on *Euryale ferox* seeds. *Asian Journal of Pharmaceutics*. 5(2):93-100.
27. **Nehal N., Mann S. and Gupta R.K.** (2015). Two promising under-utilized grains: a review. *Indian Journal of Traditional Knowledge*. 416-422.
28. **Oliveira H., Spano M., Santos C. and de Lourdes Pereira M.** (2009). Adverse effects of cadmium exposure on mouse sperm. *Reproductive Toxicology*. 28(4):550-555. https://doi.org/10.1016/j.reprotox.2009.08.001
29. **Patra R.C., Rautray A.K. and Swarup D.** (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Veterinary Medicine International*. 2011:457327. https://doi.org/10.4061/2011/457327
30. **Prakash S. and Verma A.K.** (2020). Effect of Arsenic on Serum Biochemical parameters of a fresh water cat fish, *Mystus vittatus*. *International Journal of Biological Innovations*. 2 (1): 11-19. https://doi.org/10.46505/IJBI.2020.2102
31. **Rahimzadeh M.R., Rahimzadeh M.R., Kazemi S. and Moghadamnia A.A.** (2017). Cadmium toxicity and treatment: An update. *Caspian Journal of Internal Medicine*. 8(3):135-145. 10.22088/cjim.8.3.135

32. Rathod R.V., Neve G. Jain A., and Giri P. (2023). A Comprehensive Review on Health Benefits and Nutritional Aspects of Foxnut (Makhana). *The Pharma Innovation Journal*. 12(6):4432-4438.

33. Sinha A.K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*. 47(2):389-394.

34. Sirot V., Samieri C., Volatier J.L. and Leblanc J.C. (2008). Cadmium dietary intake and biomarker data in French high seafood consumers. *Journal of Exposure Science & Environmental Epidemiology*. 18(4):400-409.

35. Song C.W., Wang S.M., Zhou L.L., Hou F.F., Wang K.J., Han Q.B., Li N. and Cheng Y.X. (2011). Isolation and identification of compounds responsible for antioxidant capacity of *Euryale ferox* seeds. *Journal of Agricultural and Food Chemistry*. 59(4):1199-1204.

36. Song J. and Wu Q.N. (2009). Analysis of therapeutic effects of Qianshi on proteinuria. *Chinese Journal of Modern Drug Application*. 3:133-134.

37. Tehseen S., Sarfraz F., Muntaham S., Ateek N., Ashfaq F., Yasmin I. and Mehmood T. (2020). Foxnut (*Euryale ferox* Salisb.): A health promising fruit. *Acta Scientific Agriculture*. 4(12):68-72.

38. Thompson J. and Bannigan J. (2008). Cadmium: toxic effects on the reproductive system and the embryo. *Reproductive Toxicology*. 25(3):304-315. https://doi.org/10.1016/j.reprotox.2008.02.001

39. Tinkov A.A., Filippini T., Ajsuvakova O.P., Skalnaya M.G., Aaseth J., Bjorklund G., Gatiatulina E.R., Popova E.V., Nemereshina O.N., Huang P.T. and Vinceti M. *et al.* (2018). Cadmium and atherosclerosis: A review of toxicological mechanisms and a meta-analysis of epidemiologic studies. *Environmental Research*. 162:240-260. https://doi.org/10.1016/j.envres.2018.01.008

40. Verma A.K. and Prakash S. (2019). Impact of Arsenic on Carbohydrate Metabolism of a fresh water cat fish, *Mystus vittatus*. *International Journal on Biological Sciences*. 10(1): 17-19.

41. Wang H.F., Chang M., Peng T.T., Yang Y., Li N., Luo T., Cheng Y.M., Zhou M.Z., Zeng X.H. and Zheng L.P. (2017). Exposure to cadmium impairs sperm functions by reducing Cat Sper in mice. *Cellular Physiology and Biochemistry*. 42(1):44-54. https://doi.org/10.1159/000477113

42. Wu C., Wang X., Wang H., Shen B., He X., Gu W. and Wu Q. (2014). Extraction optimization, isolation, preliminary structural characterization and antioxidant activities of the cell wall polysaccharides in the petioles and pedicels of Chinese herbal medicine Qian (*Euryale ferox* Salisb.). *International Journal of Biological Macromolecules*. 64:458-467. https://doi.org/10.1016/j.ijbiomac.2013.12.025

43. Yasmeen S. (2019). Cadmium induced histopathological alterations in female gonad of freshwater bivalve mollusks, *Lamellidens marginalis* during summer season. *International Journal of Biological Innovations*. 1 (2):73-77. https://doi.org/10.46505/IJBI.2019.1207

44. Yuan H., Gong Z., Meng S. and He G. (2013). Hypoglycemic and hypolipidemic effects of a triterpenoid-rich extract from *Euryale* shell on streptozotocin-induced diabetic mice. *Die Pharmazie*. 68(3):227-231. https://doi.org/10.1691/ph.2013.2734

45. Zhang C. (2009). Advance of *Euryale ferox* Salisb fundamental and application researchment. *Journal of Agricultural Technology Services*. 26(11):130-131.

46. Zhang W.N., Su R.N., Gong L.L., Yang W.W., Chen J., Yang R., Wang Y., Pan W.J., Lu Y.M. and Chen Y. (2019). Structural characterization and in vitro hypoglycemic activity of a glucan from *Euryale ferox* Salisb. seeds. *Carbohydrate Polymers*. 209:363-371. https://doi.org/10.1016/j.carbpol.2019.01.044

47. Zhao H.R., Zhao S.X., Sun C.Q. and Guillaume D. (1989). Glucosylsterols in extracts of *Euryale ferox* identified by high resolution NMR and mass spectrometry. *Journal of Lipid Research*. 30(10):1633-1637. https://doi.org/10.1016/S0022-2275(20)38246-8

48. Zhao X., Cheng Z., Zhu Y.I., Li S., Zhang L. and Luo Y. (2015). Effects of paternal cadmium exposure on the sperm quality of male rats and the neurobehavioral system of their offspring. *Experimental and Therapeutic Medicine*. 10(6):2356-2360. https://doi.org/10.3892/etm.2015.2777