Access this Article online
Quick Response Code:

Website:
jecajournal.com
Doi:
doi.org/10.4314/jeca.v21i2.5

Original Article

Influence of aqueous leave extract of *Newbouldia laevis on* cadmium chloride -induced toxicity in pituitary-testicular axis of rats

[1]Adebisi, K.A.; [1]Leko, B.; [2]Idaguko, C.A.

Abstract

BACKGROUND AND AIM: Cadmium (Cd), a natural element of the earth's crust, induce reproductive dysfunction through oxidative stress. *Newbouldia laevis* is a free radical scavenger. Our study investigated the effect of aqueous leaf extract of *Newbouldia laevis (NBL)* on cadmium chloride-induced toxicity in pituitary-testicular axis of adult male Wistar rats.

METHODOLOGY: 25 male Wistar rats weighing 130-180g were divided into 5 groups of 5 rats each; control (distilled water), 3mg/kg body weight of $CdCl_2$, 3mg/kg body weight of $CdCl_2$ + 300mg/kg body weight of NBL, 3mg/kg body weight of $CdCl_2$ + 450mg/kg body weight of NBL, 3mg/kg body weight of $CdCl_2$ + 600mg/kg body weight of NBL *Newbouldia laevis* was administered orally and a single dose of 3mg/kg body weight of $CdCl_2$ subcutaneously both for 4 weeks. Semen analysis was evaluated, also the serum was used for the oxidative stress markers, sex hormones and histoarchitecture of the testes were evaluated.

RESULTS: There was a dose-dependent effect of NBL on the semen analysis and sex hormones when compared to the $CdCl_2$ group. NBL significantly ($P<0.05$) increased SOD, CAT, GSH activities, and decreased Malondialdehyde when compared to the $CdCl_2$ group; and it was dose-dependent effect. Histologically; at the dose of *300mg/kg body weight*, there was degeneration in the seminiferous tubules, widening of interstitial spaces and testicular necrosis; while at *450mg/kg body weight, there was a* reduced portion of seminiferous epithelial diameter, no germ cells at any spermatogenic phases, cluster of Leydig cells seen in the interstitial space, degenerative changes of seminiferous tubule and reduction in the diameter of the seminiferous tubule. However, at the dose of *600 mg/kg* body weight*;* the testes revealed seminiferous tubules with increased diameters, present of Sertoli cells around the basement membrane, spermatozoa are seen in the lumen of seminiferous tubule while cluster of Leydig cells are present in the interstitial space.

CONCLUSION: *Newbouldia laevis* extracts produces a reversal of the deleterious effect of cadmium chloride on the testis at a higher dose. There is the possibility for the future use of NBL as an inhibitory agent of infertility.

Keywords:
Newbouldia laevis, *Cadmium chloride*, *Semen analysis*, *sex hormone*, *histology*.

Submitted: 25th August, 2024
Revised: 24th Ocotober, 2024
Accepted: 30th October, 2024
Published: 31st December, 2024

[1]Department of Anatomy, University of Medical Sciences, Ondo City, Ondo State, Nigeria; [2]Department of Anatomy, Faculty of Basic Medical Sciences. Edo State University Uzairue, Edo State. Nigeria.

Address for Correspondence:
Idaguko C.A.
Department of Anatomy, Faculty of Basic Medical Sciences. Edo State University Uzairue, Edo State. Nigeria.
annachi67@yahoo.com

INTRODUCTION

Fertility is impacted by environmental deterioration, which is a result of urbanization, consumerism, and progressive industrialization (Abraham and Idaguko, 2024; Idaguko and Agoreyo, 2024); 48.5 million couples worldwide suffer from infertility, a common gynecological problem (McClam *et al*., 2023). In terms of clinical terminology, it is defined as the incapacity to become pregnant after engaging in regular, unprotected sexual activity for at least a year. 50% of all cases of infertility are associated with a male causal factor, according to Huang *et al*. (2023). Accordingly, about 30% of cases of infertility are caused by male factors, and 20% are caused by a combination of male and female factors. Some individuals may have a genetic predisposition towards infertility. But nothing is known about the underlying cause of male factor infertility (Assidi, 2022).

Cadmium (Cd) is a common metallic toxicant that is present in the environment and has a negative impact on human health (Mitra *et al*., 2022). Cadmium is present in almost all foods, although the levels differ significantly depending on the type of food and level of environmental contamination (Qing *et al*., 2023). Cadmium poisoning causes nephrotoxicity, osteoporosis, cardiac problems, testicular necrosis, renal failure, ocular toxicity, and neurological abnormalities (Genchi *et al*., 2020; Charkiewicz *et al*., 2023). Several studies have shown that the testes, in particular, are especially sensitive to cadmium exposure in both human and animal reproductive

How to cite this article: Adebisi, K.A.; Leko, B.; Idaguko, C.A. Influence of aqueous leave extract of *Newbouldia laevis on* cadmium chloride -induced toxicity in pituitary-testicular axis of rats. J Exp Clin Anat 2024; 21(2): 172-179. https://dx.doi.org/10.4314/jeca.v21i2.5

systems (Abd Elhafeez *et al*., 2019; Ullah *et al*., 2023). Cadmium exposure-related male infertility is characterized by severe interstitial tissue edema, extensive necrosis of the seminiferous epithelium cells, Leydig cell damage, hemorrhage, decreased testosterone in the blood and testes, fewer germ cell junctions in the seminiferous tubules, a lack of integral membrane proteins at the Blood Testis Barrier (BTB), and decreased sperm motility and count (Massányi *et al*., 2020; Ali *et al*., 2022).

These elements cause a rise in the production of reactive oxygen species (ROS), such as hydroxyl radicals, superoxide ions, and hydrogen peroxide, which can start lipid peroxidation (Afzal *et al*., 2023). Oxidative stress is one of the main causes of the increase in male infertility patients (Takalani *et al.*, 2023). The sperm membrane is very susceptible to reactive oxygen species (ROS) peroxidation because of its high concentration of poly-unsaturated fatty acids, which could change the sperm's morphology (Emily *et al*., 2021). This is because high ROS levels can cause oxidative stress (OS) or disrupt the hormonal balance by acting on the hypothalamic axes of hormone release ROS, which in turn lower male sex hormone levels (Marwa et al., 2023). Elevated ROS can increase the risk of infertility directly or indirectly by these "endocrine disruptors," in addition to interfering with communication between the testes and the hypothalamic-pituitary unit (El-Refaei and Abdallah, 2021). This is because the hypothalamic-pituitary-gonadal (HPG) axis and other hypothalamic hormonal axes are disrupted.

Folkloric medicine has used it to cure a wide range of illnesses and afflictions, including as skin infections, toothaches, stomach aches, pains, diabetes, hypertension, cancers, malaria, and sickle cell anemia (Asase *et al*., 2010; Njoku-Oji *et al*., 2022). Hence, an increasing number of people are adopting natural plants to reduce testicular damage and reduced fertility brought on by exposure to environmental toxins (Krzastek *et al*., 2020). Originally from equatorial Africa, *Newbouldia laevis* (*NBL*) is a medium-sized angiosperm in the Bignoniaceae family that develops swiftly to become an evergreen shrub or small tree (Ogunleye *et al*., 2022). It is called the "tree of life" or "fertility tree" in Nigeria and is usually green, though the winter months do cause its leaves to turn a deep purple color. Vibrant green leaves give way to purple flowers with a delicious juice. *Newbouldia laevis* is called "Akoko" in Yoruba, "Ogirisi" in Igbo, "Aduruku" in Hausa, and "Ikhimi" in Edo (Bafor and Sanni, 2009). Extracts and compounds isolated from the leave have been reported to exhibit a wide range of biological activities, including anti-inflammatory, anti-malarial (Bafor and Sanni, 2009; Ukwubile et al., 2023). Anti-nociceptive effect (Umeyor et al., 2016) Antioxidant, hepato-renal protection, antitrypanosomal (Okagu et al., 2021), anti-hypertensive, central nervous system modulatory, and oxytocic properties (Agbafor *et al*., 2015; Enye *et al*., 2015; Bwegne *et al*., 2022). Bosha *et al*. (2019) reported that *Newbouldia laevis* leaves are utilized to treat ulcers and diarrhea. Bafor *et al*. (2014) and Kuete *et al.* (2014) have reported antipyretic and anticancer effects of the leaves.

The aim of the study was to investigate the effect of aqueous leaf extract of *Newbouldia laevis (NBL)* on cadmium chloride-induced toxicity in pituitary-testicular axis of adult male Wistar rats.

MATERIALS AND METHODS

Chemical

Cadmium chloride ($CdCl_2$ 2.5 H_2O) (Linear Formula): was obtained from Loba chemie, PVT, India and dissolved in water.

Plant harvest and extraction

The *Newbouldia laevis* (*NBL)* leaves were obtained from the University of Medical Sciences, Ondo City, in the Ondo State, Nigeria. The plant was authenticated with herbarium number UNIMED/P.B.TH/031 at the Department of Plant Science and Biotechnology of University of Medical Science, Ondo. The *NBL* leaves was air dried and pulverized. The pulverized *NBL* leaves (6kg) was subjected to Soxhlet extraction using distilled water as the solvent. Water containing the extract was then filtered and the solvent was vacuum-distilled at 4°C in a rotary evaporator. The remaining extract was finally dried in a vacuum oven at 30°C for 2 hours to ensure the removal of any residual solvent. The powdery mass yielded 80.6g, which was then stored for the study. The extract's fresh solution was prepared in distilled water.

Animals

A total of 25 Wistar adult male rats (130 to 180 gram) housed in well-ventilated rat cages in the Central Animal House, University of Medical Sciences Ondo. Ondo State. The rats were kept under standard laboratory conditions of 12-hour light and 12-hour dark cycle and were fed with standard commercial rat pellets and allowed access to water *ad libitum*. The rats were acclimatized for two weeks. The rats were weighed weekly throughout the study, the guiding principles for research involving experimental animals, as recommended by the Departmental Committee on the Use and Care of animals (National Research Council, 2011).

Experimental design

Wistar rats were divided into five groups each comprising 5 rats/ group. group I was kept as normal control. Groups II, III, IV and V received single dose subcutaneously of cadmium chloride at the dose 3 mg/kg body weight (b.wt)/per day, throughout the experiment. Group II did not receive any treatment, while the group III received 300 mg/kg b.wt of *NBL*. Group IV was treated with 450 mg/kg b.wt of *NBL* and Group V received 600 mg/kg b.wt of *NBL*. The oral administration of the extract *NBL* and cadmium chloride (single dose subcutaneously) continue for 4 weeks. At the end of the experiments, the rats were weighted and sacrificed by cervical dislocation 24 h after the last treatment. Blood was withdrawn through cardiac puncture from all groups, and serum was reserved for hormonal assay.

Hormonal assay

Serum samples were assayed for levels of follicle stimulating hormone, testosterone and luteinizing hormones using the

Microwell (solid phase) enzyme linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions and guidelines

Biochemical Assessment

Assays of Enzymatic and non-Enzymatic Anti-oxidants Catalase was estimated as described by (Choudhary *et al*., 2012). Determination of superoxide dismutase (SOD) as estimated by (Sun and Zigman, 1978). The reduced glutathione (GSH) determined by the method of (Davies *et al*., 1984). The malondialdehyde (MDA) level was determined by (D'souza *et al*., 2012)

Sperm Analysis

The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. The testicle was exposed by incising the tunica vaginalis and the caudal epididymis was harvested. The caudal epididymis of rats in each of the experimental group were removed and minced thoroughly in a specimen bottle containing normal saline for a few minutes to allow the sperms to become motile and swim out from the caudal epididymis.

Sperm count and motility studies

After 5 min incubation at 37ºC (with 5% CO_2), the semen was then taken with a 1ml pipette, dropped on a clean slide, and covered with cover slips. The slides were examined under light microscopy for sperm motility (Saalu *et al*., 2008) and with the aid of the improved Neubauer hemocytometer (Deep1/10mm LABART, Germany) counting chamber, as described by Pant & Srivastava (2003); the spermatozoa were counted under the light microscope.

Sperm morphology

This was done as described by Saalu *et al*. (2008). The sperm morphology was evaluated with the aid of a light microscope at 400x magnification. The caudal sperm was taken from the original dilution for motility, and diluted 1:20 with 10% neutral buffered formalin (Sigma- Aldrich, Canada). In wet preparations using phase contrast optics, the spermatozoa were categorized. In this study a spermatozoon was considered morphologically abnormal if it had a rudimentary tail, round or detached head and was expressed as a percentage of morphologically normal sperm.

Testicular histology preparation

The testes were collected and kept in 10% neutral buffered formalin. The tissues were dehydrated, infiltration, embedded, and cut using rotary microtome at 5 microns. Sections of 5-micron thickness were prepared and stained by hematoxylin and eosin (H&E) and examined by light microscope

Data presentation and statistical analysis

All statistical analyses were done using power BI, Graph pad prism (version 8.0.3). Results were presented as mean ± Standard Error of Mean (S.E.M). The groups were compared using Analysis of variance (ANOVA) and followed by Bonferroni post hoc- tests. Differences were considered significant when is taken as $p<0.05$.

RESULTS

Epididymal sperm characteristics

For $CdCl_2$ group, the sperm count ($13.8 \pm 0.8 \times 10^6$) was significantly decreased ($P < 0.05$) compared to control group ($130.1 \pm 0.7 \times 10^6$). However, in $CdCl_2$ + 300mg of NBL ($25.7 \pm 0.4 \times 10^6$), $CdCl_2$ + 450mg of NBL ($30.4 \pm 0.3 \times 10^6$), $CdCl_2$ + 600mg of *NBL* ($37.2 \pm 0.8 \times 10^6$) groups, sperm count was significantly increased when compared to $CdCl_2$ group ($13.8 \pm 0.8 \times 10^6$). Percentage of the progressive sperm movement for $CdCl_2$ group (20.00 ± 0.01) showed a significant decrease ($P <0.05$) compared to the control group (90.67 ± 5.36). There was a significantly increased ($p<0.05$) in the $CdCl_2$ + 450mg of NBL (52.00 ± 2.89) and $CdCl_2$ + 600mg of NBL (60.30 ± 1.20) respectively when compared to $CdCl_2$ group. For non-progressive movement, there was significant increase in $CdCl_2$ group (37.67 ± 3.33) compared to the control (5.03 ± 4.41), however, there was no significant decease in non-progressive movement of sperm cells in $CdCl_2$ + 300mg of NBL, $CdCl_2$ + 450mg of NBL, $CdCl_2$ + 600mg of *NBL* when compared to the $CdCl_2$ group. The immotile sperm was significantly increased in $CdCl_2$ group (43.10 ± 3.33) when compared to the control group (5.00 ± 4.00); however, there was dose-dependent significant decrease in all the treated groups compared to $CdCl_2$ group (Table 1).

Evaluation of sperm morphology

Analysis of normal morphology of the semen samples revealed a significant decrease ($P<0.05$) in $CdCl_2$ group when compared with control group. However, there was a dose-dependent significant increase in the normal morphology in $CdCl_2$ + 300mg of *NBL*, $CdCl_2$ + 450mg of *NBL* and $CdCl_2$ + 600mg of *NBL* groups when compared to $CdCl_2$ group. There was a significantly increased ($P<0.05$) incidence of sperm with abnormal head, neck/mid-piece and tail defects in $CdCl_2$ group when compared to the control group. Exposure to $CdCl_2$ and *NBL* produce significantly decreased ($P<0.05$) in the incidence of sperm with abnormal head, neck/mid-piece and tail defects in all the treated groups, which was dose-dependent when compared to the $CdCl_2$ group (Table 2).

Hormonal Profile

There were statistically significant decrease ($P <0.05$) in the FSH, LH and testosterone of the $CdCl_2$ group compared to control group. However, there were statistically significant increases ($P <0.05$) in FSH and LH and testosterone of all the treated groups (300, 450 and 600 mg/kg b.wt of the extract) when compared to the $CdCl_2$ group (Table 3).

Biochemical Assessment

There was statistically significant decrease ($P <0.05$) in GSH in the $CdCl_2$ group compared to the control. There was no significant

different (P <0.05) in 300 and 450 mg/kg b.wt of *NBL* when compared to $CdCl_2$ group. However, there was statistically significant increased (P <0.05) in 600 mg/kg b.wt of *NBL* compared to $CdCl_2$ group. There was statistically significant decrease (P <0.05) in the catalase of the $CdCl_2$ group compared to the control group; however, there was dose-dependent significant increase (P <0.05), in all the treatment groups (300, 450 and 600 mg/kg b.wt of *NBL*) compared to $CdCl_2$ group. There was statistically significant decrease (P <0.05) in superoxide dismutase of the $CdCl_2$ group compared to the control group, however; there was statistically significant increase in the groups (450 mg and 600 mg /kg b.wt of *NBL)* compare to the $CdCl_2$ group. There was statistically significant increase (P<0.05) in the MDA of $CdCl_2$ group compared to the control group. However; there were significant decrease (P<0.05) in the groups (450 mg and 600mg/ kg b.wt of *NBL*) compared to $CdCl_2$ group (Table 4).

Histology of the testes

The control group photomicrograph showed a thick fibrous tissue capsule, the seminiferous tubules indicated normal structure, which were lined by spermatogonia, followed by successive layers of germinal epithelium at different stages of spermatogenesis with normal supporting Sertoli cells as seen in figures 4 (Control). As shown in figure 4 for $CdCl_2$ group, the seminiferous tubules with degenerative changes; closure of interstitial spaces and edematous changes, separation of basement membrane from underlying layer with infiltrations by mononuclear cells and destruction of the seminiferous epithelium. The figure 4 of $CdCl_2$ and 300mg/kg group, showed area of degeneration in the seminiferous tubules, widening of interstitial spaces and testicular necrosis. Figure 4 of $CdCl_2$ and 450mg/kg group, showed reduced portion of seminiferous epithelial diameter, no germ cells at any spermatogenic phases, cluster of Leydig cells seen in the interstitial space, degenerative changes of seminiferous tubule and reduction in the diameter of the seminiferous tubule. While widening of the interstitial space, clusters of Leydig cell, improved seminiferous tubules, increased seminiferous tubule diameters, present of Sertoli cells around the basement membrane, spermatozoa are seen in the lumen of seminiferous tubule cells as seen in figure 1 of $CdCl_2$ and 600mg/kg group.

Table 1: The effect of aqueous leaf extracts of *NBL* on sperm parameters of $CdCl_2$ induced toxicity in adult male Wistar rat

GROUPS	Sperm count X (10^6)/ml)	Progressive X (10^6)/ml)	Non progressive X (10^6)/ml)	Immotile X (10^6)/ml)
Control	130.10 ± 0.71	90.67±5.36	5.03±4.41	5.00±4.00
$CdCl_2$ only (3mg)	13.81 ± 0.81*	20.00±0.01*	37.67±3.33*	43.10±3.33*
$CdCl_2$ (3mg) then NBL (300mg)	25.70± 0.44*#	29.20±0.01*	33.00±2.89*	38.00±2.89*#
$CdCl_2$ (3mg) then NBL (450mg)	30.41±0.33*#	52.00±2.89*#	31.00±2.89 *	17.00±5.77*#
$CdCl_2$ (3mg) then NBL (600mg)	37.20±0.81*#	62.30±1.20*#	30.00±1.00*	8.70±2.19#

Value expressed as mean ± Standard Error of Mean (S.E.M). * Significantly different from control (P < 0.05), # Significantly different from $CdCl_2$ only (P < 0.05)

Table 2: The effect of aqueous leaf extracts of *NBL* on sperm morphology analysis (%) of $CdCl_2$ induced toxicity in adult male Wistar rats

GROUPS	Normal Morphology (%)	Head Defects (%)	Neck/Mid-piece Defects (%)	Tail Defects (%)
Control	82.33±1.45	5.067±1.67	6.237±1.67	6.112±1.67
$CdCl_2$ only (3mg)	30.65±0.33*	26.03±1.67*	22.00±1.67*	21.33±1.67*
$CdCl_2$ (3mg) then NBL (300mg)	46.03±1.67*#	21.31±3.33*	18.23±1.67 *	14.10±2.89 *
$CdCl_2$ (3mg) then NBL (450mg)	53.62±1.67*#	20.20±1.67*	16.10±3.33*	10.14±2.87
$CdCl_2$ (3mg) then NBL (600mg)	70.33±1.67*#	11.00±0.01	11.12±0.01	8.00±1.67

The data are expressed as Mean ± SEM, N=5; * Significantly different from control (P < 0.05); # Significantly different from $CdCl_2$ only (P < 0.05)

Table 3: The effect of aqueous leaf extracts of *NBL* on hormonal profile of $CdCl_2$ induced toxicity in adult male Wistar rats.

GROUPS	FSH (miU/ml)	LH (miU/ml)	TESTOSTERONE (ng/ml)
Control	6.80±0.03	7.06±0.07	5.91±0.04
$CdCl_2$ only (3mg)	3.19± 0.05*	3.67±0.05*	0.44±0.02*
$CdCl_2$ (3mg) then NBL (300mg)	6.35±0.02#	5.79± 0.07#	3.14±0.07#
$CdCl_2$ (3mg) then NBL (450mg)	6.43±0.01#	5.76±0.01#	3.82±0.09#
$CdCl_2$ (3mg) then NBL (600mg)	6.55±0.03#	6.88±0.01#	4.95±0.04#

* Significantly different from control (P < 0.05), # Significantly different from $CdCl_2$ only (P < 0.05)

Table 4: The effect of aqueous leaf extracts of *NBL* on $CdCl_2$ induced toxicity in adult male Wistar rats on antioxidants and MDA.

GROUPS	MDA (U/mg protein)	SOD (U/mg protein)	CATALASE (U/mg protein)	GSH (U/mg protein)
Control	0.11±0.00	60.20± 2.62	218.60±2.19	14.50±0.93
$CdCl_2$ only (3mg)	1.57±0.01*	33.31± 2.32*	185.20±7.40*	10.09±0.71*
$CdCl_2$ (3mg) then NBL (300mg)	1.15±0.01	40.79 ±2.86	205.80±5.82#	11.18±0.82
$CdCl_2$ (3mg) then NBL (450mg)	0.14±0.01#	51.51±1.12#	213.20±3.92#	11.52±0.16
$CdCl_2$ (3mg) then NBL (600mg)	0.13±0.01#	58.53±3.42#	221.50±7.52#	13.88±0.09#

* Significantly different from control ($P < 0.05$); # Significantly different from $CdCl_2$ only ($P < 0.05$)

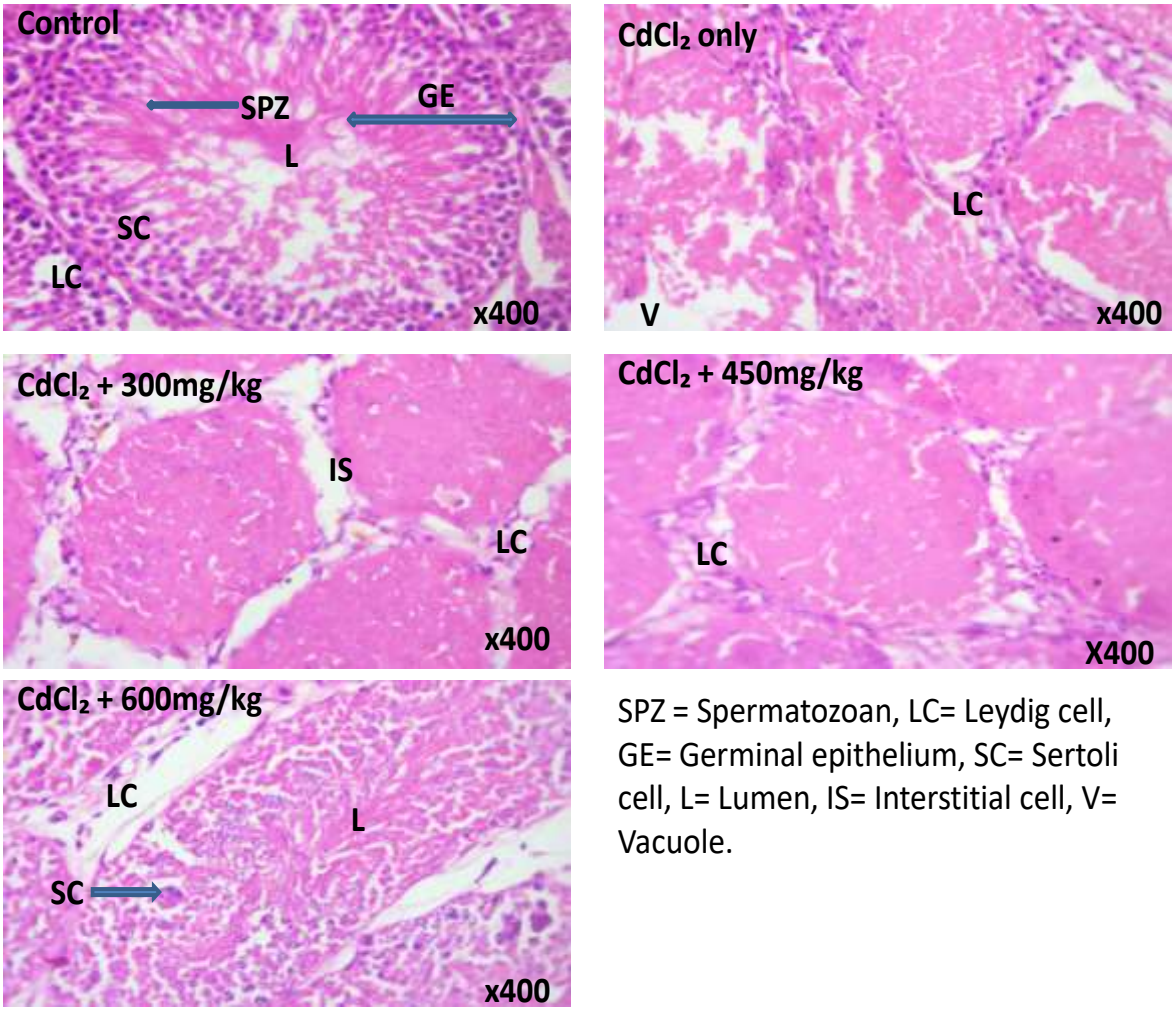

Figure 1: Representative photomicrograph of a section of testis morphology stained with H&E x400 magnification

DISCUSSION

According to the current study, sperm motility and count were significantly reduced by $CdCl_2$, while abnormal sperm morphology increased. These observations may be the result of $CdCl_2$'s detrimental effects on spermatogenesis. In the seminiferous tubules, cadmium significantly decreases primary and secondary spermatocytes, according to several studies (Zhu *et al*., 2020; Qi *et al*., 2023). Rajput *et al*. (2021) and Jun *et al*. (2023) state that superoxide dismutase is an antioxidant that lowers free oxygen radical to non-toxic molecules, whereas glutathione reductase turns hydrogen peroxide into a non-toxic product. Exposure to cadmium may change these biological enzymes. Numerous biochemical enzymes, such as glutathione reductase, catalase, and superoxide dismutase, were found in low concentrations in the rat given $CdCl_2$. these findings are in line with the report of Erdem *et al*. (2016).

However, this effect was mitigated by the *NBL* dosages of 450 mg and 600 mg. This illustrated the protective nature of the extract.

Sperm are particularly susceptible to lipid peroxidation because of the high concentration of unsaturated fatty acids in their plasma membrane and the deficiency of cytoplasmic antioxidants (Gualtieri *et al*., 2021). The results of the current sperm profile matched the findings (Al *et al*., 2022) that shown cadmium can cause lipid peroxidation in rats. Conversely, there was a significant ($p<0.05$) decrease in malondialdehyde levels in the plant extract group. The leaf extract has a high phenol content and a high concentration of vitamin C, which, according to Salemcity *et al*. (2020), indicates that it has good antioxidant activity that can inhibit lipid peroxidation.

According to reports, cadmium causes neurotoxicity, which leads to an imbalance in hormones. The lower levels of testosterone, luteinizing hormone (LH), and FSH in the $CdCl_2$ group may be explained by this. The hypothalamus, which secretes the hormone that releases gonadotropin, is another organ affected by cadmium (Antar *et al*., 2024). FSH, LH, and testosterone increased in the leaf extract of the *NBL* treated groups, but not as much as in the control group. Consequently, the adverse effects of $CdCl_2$ on semen profile may potentially be due to a reduction in testosterone levels or the generation of reactive oxygen species.

The histology result indicates that cadmium causes considerable testicular degeneration and seminiferous tubule damage, which is consistent with other research showing that cadmium can cause testicular tissue necrosis and apoptosis in rats (Behairy *et al*., 2024). At lower dosages of 300 mg/kg and 450 mg/kg, NBL was unable to mitigate the testicular damage caused by $CdCl_2$, but at a higher dose of 600 mg/kg, the seminiferous tubule displayed evidence of nearly normal repair, including the formation of Sertoli cells and Leydig cells. Several studies have demonstrated that *NBL* is a potent antioxidant due to its many antioxidant properties (Osigwe *et al.,* 2017; Rashed, 2021).

Furthermore, studies have shown that *NBL* contains anthraquinone, flavanoids, pyrazole alkaloids, saponins, and glycosides (Rashed, 2021). The results of the study show that exposure to $CdCl_2$ causes significant damage to the testis because it increases oxidative stress, disrupts hormone balance, and alters the histology of the testicles. However, *NBL* dosages of 300 and 450 mg/kg body weight demonstrated no structural evidence of protective effect against testicular toxicity induced by $CdCl_2$. Larger dosages exceeding 600 mg/kg body weight are therefore anticipated.

Conclusion: The present study demonstrates that cadmium chloride impairs sperm quality, and can reduce male fertility potential. *Newbouldia laevis* aqueous leaf extracts at a higher dose improved testicular toxicity caused by cadmium chloride. There is the possibility for the future use of NBL as an inhibitory agent of infertility

Declaration of Conflicting Interests: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Acknowledgments: The authors would like to express our special thanks to The Management of University of Medical Science, Ondo for the technical assistance rendered during the preparing of the study.

Authors' contribution: LB and AKA has planned, designed, completed the statistical analysis and wrote the manuscript. AKA carried out the experiment work and the Laboratory tests. All the work of this article was under the supervision of LB and ICA, all the authors read and approved the final manuscript

REFERENCES

Abd Elhafeez, EM., Halawa, AA., Hamed, MF., Abouelmaged, MM. (2019). Effects of Cadmium and /or Chromium on reproductive organs and semen profiles of male albino rats. Mansoura Veterinary Medical Journal; 20(3): 14-18. https://doi.org/10.21608/mvmj.2019.23.203

Abraham, A., Idaguko CA (2024). Protective Effects of Tigernut (Cyperus esculentus) on Bisphenol A- Induced Testicular Toxicity in Wistar Rats. International Journal of Medical Surgical Sciences. 11(2): 1-14. https://doi.org/10.32457/ijmss.v11i2.2588

Afzal, S., Abdul Manap, AS., Attiq, A., Albokhadaim, I., Kandeel, M., Alhojaily, SM. (2023). From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. Front Pharmacol; 18;14:1269581. https://doi: 10.3389/fphar.2023.1269581.

Agbafor, K., Ezeali, C., Akubugwo, E., Obiudu, I., Uraku, A., Ogbanshi, M, et al. (2015). Cardioprotective Effect of Leaf and Root Extracts of Newbouldia laevis against Carbon Tetrachloride Induced-Cardiotoxicity in Albino Rats. European Journal of Medicinal Plants; 9:1-7. https:// doi: 10.9734/EJMP/2015/17673

Ali, W., Ma, Y., Zhu, J., Zou, H., Liu, Z. (2022). Mechanisms of Cadmium-Induced Testicular Injury: A Risk to Male Fertility. Cells; 14;11(22):3601. https://doi: 10.3390/cells11223601.

Antar, SA., Halouani, A., Gad, C., Al-Karmalawy, AA. (2024). An Overview of the Mechanisms of Cadmium-Induced Toxicity in the Male Reproductive System. Pharmaceutical Sciences; 30(1), 1-17. https://doi: 10.34172/PS.2023.10

Asase, A., Akwetey, GA., Achel, DIG. (2010). Ethnopharmacological use of herbal remedies for the treatment of malaria in Dangme West district of Ghana. Journal of Ethnopharmacology; 129:367-376. https:// doi: 10.1016/j.jep.2010.04.001

Assidi, M. (2022). Infertility in Men: Advances towards a Comprehensive and Integrative Strategy for Precision Theranostics. Cells; 22;11(10):1711. https://doi: 10.3390/cells11101711.

Bafor, E., Sanni, U. (2009). Uterine contractile effects of the aqueous and ethanol leaf extracts of Newbouldia Laevis (Bignoniaceae) in vitro. Indian J Pharm Sci; 71(2):124-127. https://doi: 10.4103/0250-474X.54274

Bafor, EE., Uwumarongie, OH., Omoregbe, A. (2014). Antipyretic activity of orally administered extracts of Newbouldia laevis (Bignonaceae) in mice. J Sci Pract Pharm; 1(1):7-10.

Behairy, A., Hashem, MMM., Abo-EL-Sooud, K., Soliman, AM., Mouneir, SM., El-Metwally, AE., Ismail, SH., Hassan, BA., Abd-Elhakim, YM. (2024). Influence of titanium dioxide nanoparticles and/or cadmium chloride oral exposure on testicular morphology, oxidative stress, and apoptosis in rats: Ameliorative role of co-enzyme Q10. Heliyon 10(1), E24049, DOI:https://doi.org/10.1016/j.heliyon.

Bosha, JA., Sule, S., Asuzu, IU. (2019). Antidiabetic and hypolipidemic effects of methanol leaf extract of Newbouldia laevis in alloxan induced diabetic rats. Adv Res Gastroentero Hepatol; 13(3):56-62. https://doi:10.19080/ARGH.2019.13.555863.

Bwegne JGBN., Ayina, CAN., Pagnangdé, AHBW., Etaga, NB., Nzekuie, QS, Ouedraogo, MS., Mandengue, SH., Minkande, JZ. (2022). Newbouldia's Laevis biological effects against metabolic syndrome components: A review. World Journal of Advanced Research and Reviews, 16(02), 121–138

Charkiewicz, AE., Omeljaniuk, WJ., Nowak, K., Garley, M., Nikliński, J. (2023). Cadmium Toxicity and Health Effects—A Brief Summary. Molecules; 28(18):6620. https://doi.org/10.3390/molecules28186620

Choudhary, R, Saroha AE, Swarnkar PL. (2012). Effect of abscisic acid and hydrogen peroxide on antioxidant enzymes in Syzygium cumini plant. J Food Sci Technol. 49(5):649–52. https://doi: 10.1007/s13197-011-0464-3

Davies, H., Birt, DF., Schnell, RC. (1984) Direct enzymatic assay for reduced and oxidized glutathione. Pharmacol Methods. 12(3):191–194. https://doi: 10.1016/0160-5402(84)90059-7

D'souza, D., Subhas, BG., Shetty, SR., Balan P (2012). Estimation of serum malondialdehyde in potentially malignant disorders and post-antioxidant treated patients: A biochemical study. Contemp Clin Dent. 3(4):448–451. https://doi: 10.4103/0976-237X.107438

El-Refaei, MF., Abdallah, EAA. (2021). Protective effects of caffeic acid phenethyl ester on cadmium-induced testicular injury: A crucial role of antioxidant enzymes in male mice infertility. Heliyon; 7(5):e06965. https://doi: 10.1016/j.heliyon.2021.

Emily, PP., Evans, JTM., Scholten, AM., Claudia RSan-M., Arturo, EL, Thamir H., Eus, GJM., Arts, RS., Astrid, E.P.C. (2021). Male subfertility and oxidative stress, Redox Biology; 46:102071, I2213-2317,

Enye, LA., Edem, EE., Gonaka, MG., Ekundina, V., Akingbade, AM., Adefisayo, MA. (2015). The effect of Newbouldia laevis on wistar rat's testis and liver histomorphology in cadmium-induced testicular toxicity and hepatotoxicity. World Journal of Pharmacology and Pharmaceutical Science; 4(11):100-08.

Erdem, O., Yazihan, N., Kocak, MK., Sayal, A., Akcil, E. (2016). Influence of chronic cadmium exposure on the tissue distribution of copper and zinc and oxidative stress parameters in rats. Toxicology and Industrial Health; 32(8):1505-1514. https://doi:10.1177/0748233714566875

Genchi, G., Sinicropi, MS., Lauria, G., Carocci, A., Catalano, A. (2020). The Effects of Cadmium Toxicity. International Journal of Environmental Research and Public Health; 17(11):3782. https://doi.org/10.3390/ijerph17113782

Gualtieri, R., Kalthur, G., Barbato, V., Longobardi, S., Di Rella, F., Adiga, SK., Talevi, R. (2021). Sperm Oxidative Stress during In Vitro Manipulation and Its Effects on Sperm Function and Embryo Development. Antioxidants (Basel); 10(7):1025. https://doi: 10.3390/antiox10071025.

Huang, B., Wang, Z., Kong, Y. *et al.* (2023). Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: an analysis of global burden of disease study. BMC Public Health; 23, 2195. https://doi.org/10.1186/s12889-023-16793-3

Idaguko, CA., Agoreyo, G. (2024). Effect of Wheat (Triticum aestivum Linn.) Diet on the Testes of Sprague-Dawley Rats. Balkan Med J; https:// doi: 2024-2-6710.4274/balkanmedj.galenos.

Jun, P., Xingyu, P., Guanghui, W., Yi, H. (2023). Research progress of glutathione peroxidase family (GPX) in redoxidation. Front. Pharmacol; 14. https://doi.org/10.3389/fphar.2023.1147414.

Krzastek, SC., Farhi, J., Gray, M., Smith, RP. (2020). Impact of environmental toxin exposure on male fertility potential. Transl Androl Urol; 9(6):2797-2813. https://doi: 10.21037/tau-20-685

Kuete, V., Tankeo, SB., Saeed, MEM., Wiench, B., Tane, P., Efferth, T. (2014). Cytotoxicity and modes of action of five Cameroonian medicinal plants against multi-factorial drug resistance of tumor cells. J. Ethnopharmacol; 153:207-19. https:// doi:10.1016/j.jep.2014.02.025.

Marwa, L., Henda M., Véronique, B., Hafida, K-C., Moncef, B., et al.. (2023). Oxidative stress in male infertility and therapeutic approach: A mini-review. Asian Pacific Journal of Reproduction; 12 (6):249-255.

Massányi, P., Massányi, M., Madeddu, R., Stawarz, R., Lukáč, N. (2020). Effects of Cadmium, Lead, and Mercury on the Structure and Function of Reproductive Organs. Toxics; 8(4):94

McClam, M., Liu, J., Fan, Y. *et al.* (2023). Associations between exposure to cadmium, lead, mercury and mixtures and women's infertility and long-term amenorrhea. Arch Public Health; 81, 161. https://doi.org/10.1186/s13690-023-01172-6

Mitra, S., Chakraborty AI., Tareq, AM., Emran, TB., Nainu, F., Khusro, A., Idris, AM., Khandaker, MU., Osman, H., Alhumaydhi, FA., Simal-Gandara, J. (2022). Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University - Science; 34:3, 101865.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. (2011) 8th edition. Washington (DC): National Academies Press (US). Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050/ https://doi: 10.17226/12910

Njoku-Oji, NN., Ifegwu, NO., Igbonaju, M., Okwuonu, IF., Chukwukaeme, CW., Mmaju, CI., Okonudo, PO. (2022). Effect of Ethanoic Leaf Extracts of Carica Papaya and New Bouldia Laevis on Lipid Profile of Diabetic Wistar Rats. International Journal of Research and Scientific Innovation (IJRSI); |9(2). 2321-2705

Ogunleye, MB., Aina, KS., Adeniyi, IM (2022). Assessment on Anatomical and Physico-Mechanical Properties of Grown Newbouldia Laevis (P. BEAUV.) and Aningeria Robusta (AUBREV. AND PELLEGR.) in Nigeria for Utilization Potentials. RJOAS; 2(122). UDC 633; https://doi 10.18551/rjoas.2022-02.13

Okagu, IU., Ndefo, JN., Agbo, MO. (2021). Trado-Medical uses, Chemical Constituents and Biological Activities of Newbouldia laevis (Bignoniaceae): A Review, Pharm Sci., doi: 10.34172/PS.2021.29

Osigwe, CC., Akah, PA., Nworu, CS., Okoye, FBC. (2017). Apigenin: A methanol fraction component of Newbouldia laevis leaf, as a potential antidiabetic agent. J Phytopharmacol; 6(1):38-44.

Qi, Z., Wei, X., ZiYu, K., YuJiao, W., Yi, Liu. (2023). Cadmium exposure-induced rat testicular dysfunction and its mechanism of chronic stress. Food and Chemical Toxicology; 182:114181. https://doi: 10.1016/j.fct.2023

Qing, Y., Li, Y., Cai, X. et al. (2023). Assessment of Cadmium Concentrations in Foodstuffs and Dietary Exposure Risk Across China: A Metadata Analysis. Expo Health; 15, 951–961. https://doi.org/10.1007/s12403-022-00530-z

Pant and Srivastava (2003). Testicular and spermatotoxic effects of quinalphos in rats. J Appl. Toxicol; 23(4): 271-274. https://doi:10.1002/jat.919.

Rajput, VD., Harish, SRK., Verma, KK., Sharma, L., Quiroz-Figueroa, FR., Meena, M., Gour, VS., Minkina, T., Sushkova, S., et al. (2021). Recent Developments in Enzymatic Antioxidant Defence Mechanism in Plants with Special Reference to Abiotic Stress. *Biology*; 10(4):267. https://doi.org/10.3390/biology10040267

Rashed, K., (2021). Phytochemical and biological effects of Newbouldia laevis: Areview. Plantae Scientia; 4(5):208-213. https://DOI:10.32439/ps.v4i5.208-213

Saalu, LC., Udeh, R., Oluyemi, KA., Jewo, PI., Fadeyibi, LO. (2008). The ameroriating effects of grapefruit seed extract on testicular morphology and function of varicocelized rats. Int. J. Morphol; 26(4): 1059-1064.

Salemcity, AJ., Nwaneri-Chidozie, VO., Adameh, E., Effiong, ME (2020). Antioxidant and Free Radical Scavenging Activities of Newbouldia laevis Leaf extracts. Free Radical Antioxidant; 10(1):10-15. https://doi.org/10.5530/fra.2020.1.3

Sun, M., Zigman S. (1978). An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Biochem. 90(1):81–89. https://doi:10.1016/0003-2697(78)90010-6

Takalani, NB., Monageng, EM., Mohlala, K., Monsees, TK., Henkel, R., Opuwari, CS. (2023). Role of oxidative stress in male infertility. Reproduction and Fertility; 4(3). e230024. https://doi: 10.1530/RAF-23-0024.

Ukwubile, CA., Ikpefan, EO., Dibal, MY., Umeano, VA., Menkiti, DN., Kaosi, CC., Paul, S., Famurewa, AC., Nettey, H., Yerima, TS. (2023). Pharmacognostic profiles, evaluation of analgesic, anti-inflammatory and anticonvulsant activities of Newbouldia laevis (P. Beauv.) Seem. Ex Bureau leaf and root extracts in Wistar rats. J Ethnopharmacol 5;314:116632. https://doi: 10.1016/j.jep.2023.

Ullah, S., Ennab, W., Wei, Q., Wang, C., Quddus, A., Mustafa, S., Hadi, T., Mao, D., Shi, F. (2023). Impact of Cadmium and Lead Exposure on Camel Testicular Function: Environmental Contamination and Reproductive Health. Animals; 13(14):2302. https://doi.org/10.3390/ani13142302

Umeyor, C., Anaka, E., Kenechukwu, F., Agbo, C., Attama, A. (2016). Development, in vitro and in vivo evaluations of novel lipid drug delivery system of *Newbouldia laevis* (P. Beauv.). Nanobiomedicine (Rij). 22;3:1849543516673445. https://doi: 10.1177/1849543516673445.

Zhu, Q., Li, X., Ge, RS. (2020). Toxicological Effects of Cadmium on Mammalian Testis. Front Genet; 26(11): 527. https:// doi: 10.3389/fgene.2020.00527.