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# ANTI-TUMOURIC CAPACITY `OF *Uvaria chamae* ROOT EXTRACT ON CADMIUM-INDUCED TESTICULAR SOLID MASS TUMOUR IN WISTAR RATS

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## ABSTRACT

This study was carried out to investigate the efficacy of *Uvaria chamae* root extract on cadmium-induced testicular solid mass tumour. Twenty-five adult male Wistar rats were used and the experiment lasted for 8_weeks. The rats were divided into five groups of five animals each namely: Group one (control) were fed with a normal diet and distilled water, Group two; rats were administered 3_mg/kg of cadmium only, Group three; rats were administered 3_mg/kg of cadmium and treated with 500_mg/kg of *Uvaria chamae* root extract, Group four; rats were administered 3_mg/kg of cadmium and treated with 1000_mg/kg of *Uvaria chamae* root extract and Group five were administered 3mg/kg of cadmium and treated with 1500_mg/kg of *Uvaria chamae* root extract. After the experiment, the rats were anaesthetized, sacrificed and the testes were harvested. Testicular tissues were immediately fixed in 10% buffered formalin, processed and stained with Hematoxylin and Eosin (H&E) staining method. From our findings, Group one showed normal testicular histo-morphology, Group 2 showed interstitial connective tissue degeneration, detachment of the seminiferous tubules from their support, Leydig cells and spermatogenic cell necrosis and formation of solid masses of tumors within the lumen of the seminiferous tubules, Group 3 showed rejuvenation of the interstitial connective tissues with persistent Leydig cells necrosis and continuous formation of solid masses of tumors within the lumen of the seminiferous tubules, Group four showed rejuvenation of the interstitial connective tissue at several regions within the testis, presence of spermatogenic cells, disappearance and reduction of the solid masses of tumour in majority of the seminiferous tubules but with few tumor-like growth within few seminiferous tubules, Group 5 revealed rejuvenation of the interstitial connective tissue at several regions within the testis, presence of spermatogenic cells and disappearance and reduction of the solid masses of tumor in majority of the seminiferous tubules. Following these results, it is safe to say that *Uvaria chamae* root extract can ameliorate the effects of cadmium-induced testicular tumours on the testes.

**KEYWORDS:** Cadmium, testes, testicular tumor, *Uvaria chamae*, histo-morphology, Wistar rats

## INTRODUCTION

The male reproductive system is an essential part of total human health since it helps to ensure the survival of the species and the welfare of each individual. However, the modern environment offers serious risks to the health of male reproduction, with exposure to heavy metals like cadmium emerging as a major worry. A common environmental contaminant known for its harmful effects on many organs, particularly the testes, is cadmium. Male infertility may be due to the result of cadmium-induced testicular toxicity, which is characterized by decreased spermatogenesis, reduced semen quality, and hormonal abnormalities. Known to have therapeutic benefits, *Uvaria chamae*, has historically been used as a potential treatment for some toxicity related diseases (Edem *et al*., 2024a). It is thought to have anti-inflammatory, cytoprotective, and antioxidant effects (Edem *et al*., 2023b). The specific therapeutic properties of *Uvaria chamae* against cadmium-induced testicular toxicity, and more importantly, its influence on testicular quality, remains poorly understood despite this longstanding use. Due to its possible therapeutic characteristics, such as its antioxidant and anti-inflammatory actions, it has a long history of usage in traditional medicine. According to certain studies, *Uvaria chamae* may act as a remedy a range of toxic impediments, including heavy metal poisoning (Edem *et al*., 2024b). Regarding its precise protective and ameliorative effects on the male reproductive system, there is, however, little scientific evidence, particularly in the context of cadmium-induced testicular solid mass tumor. Cadmium is a chemical element with the atomic number 48 and the symbol Cd. It is a relatively rare and hazardous heavy metal that is toxic to humans and animals. It is largely available in the environment (Benoff, 2009). It is a widespread environmental contaminant found in various sources, including industrial emissions, tobacco smoke, contaminated food and water. Once absorbed into the body, cadmium amasses in various tissues, with a particular predilection for the testes. Cadmium exposure has been linked to testicular impairment, characterized by reduced sperm production, altered sperm morphology, and impaired sperm motility. Testicular toxicity by cadmium inducement can disrupt hormone balance, including reduced testosterone levels, leading to infertility and reproductive health issues in males (Godt *et al*., 2006).

## MATERIALS AND METHODS

### Materials Used

The materials used for this research included twenty-five (25) adult male Wistar rats, clean wooden cages, saw dust, feed bowls, water bowls, vital feeds, masking tape, permanent markers, electrical weighing balance, iodine, insulin syringe, dissecting set, dissecting board, beaker, distilled water, cadmium, *Uvaria chamae,* buffered formalin, small and large sized plain sample bottles.

**Plant Acquisition**

*Uvaria chamae* roots were harvested from a local farm in Ikot Efre Itak in Ikono Local Government Area, Akwa Ibom State, Nigeria.

**Plant Extraction**

After the roots were harvested, they were washed thoroughly and air dried for few days to make sure that all unwanted moisture were removed. The roots were carefully grinded using an electric blender and macerated with 2 litres of 70% alcohol and then kept for a period of 72_hours and then filtered with the use of a filter paper. The filtrate was them kept in a water bath until evaporation takes place. About 134_g of the dry matter of the extract and stored in a refrigerator for the experiment.

**Animal Management and Protocol**

In this research, the weight of the 25-adult male albino Wistar rats used were between 143_g and 342_g. The rats were purchased from the Department of Pharmacology and Toxicology, Faculty of Pharmacy Animal House and were acclimatized for two weeks at the Faculty of Basic Medical Sciences Animal House before the commencement of administration. The animals were kept in wooden cages with sawdust as beddings under suitable weather conditions. The animals were given normal rat meal and water. The animals were divided into five groups of five animals each, with group one being the control and group two through five being the experimental groups. All animals were handled in accordance with the National Academy of Science's "guide for the care and use of laboratory animals" which was published by the National Institute of Health.

**Acclimatization of Experimental Animals**

The animals were sheltered in wooden cages and acclimatized for two weeks (14_days) in the Faculty of Basic Medical Sciences' Animal House, University of Uyo, which is situated at the University of Uyo, Annex Campus, Uyo. Before the experimental phase kicked off, the animals went through this acclimatization to help them adapt to their new surroundings. During the period of acclimatization and the entire period of study, the animals were given unlimited access to water and feed. The animals were managed in strict accordance with the university's animal handling ethics.

**Intervention/Administration of Cadmium and *Uvaria chamae***

*Uvaria chamae* root extract was administered orally while cadmium (Cd) was administered intraperitoneally with relation to the body weight of the experimental animals. Cadmium administration which commenced immediately after acclimatization was once a week and the dosage was 3_mg/kg of cadmium except the control group. The substance (cadmium) was administered using an insulin syringe.

The animals were divided into five groups of 5 rats per group. The groups were arranged thus: Group 1 (control) were fed on normal diet and distilled water; Group 2 (Cd only) were induced with cadmium with the dosage of 3mg/kg body weight; Group 3 (Cd and *Uvaria chamae*) were induced with 3_mg/kg of Cd and then treated with of 500_mg/kg of extract; Group 4 (Cd and *Uvaria chamae*) were induced with 3_mg/kg of Cd and then treated with 1000_mg/kg of extract; Group 5 (Cd plus *Uvaria chamae*) were induced with 3_mg/kg of Cd and then treated with 1500_mg/kg of extract as shown in table 1. Testicular tissues were collected for subsequent histological.

**Experimental Design**

Table 1: Table showing Experimental Design

| Groups | Cadmium Administered mg/kg | Extract Administered mg/kg | Remarks |
|---|---|---|---|
| 1 | Nil | Nil | Control |
| 2 | 3 | Nil | Cadmium Alone |
| 3 | 3 | 500 | Cadmium and extract |
| 4 | 3 | 1000 | Cadmium and extract |
| 5 | 3 | 1500 | Cadmium and extract |

**Sacrifice and Organ Extraction**

After the administration for the period of four (4) weeks, all the animals were scarified under anesthesia (chloroform) and the testicular tissues were collected from each animal. The rats were put into a transparent jar that was laced with chloroform (a colorless liquid that evaporates into gas) and its effects include shortness of breath and irritation of the throat and nose. The testes were harvested immediately and put in a big plain sample bottle and were fixed with 10% buffered formalin. The sample bottles containing the tissues were sent to the Histology preparatory room in the Department of Human Anatomy, University of Uyo for tissue processing.

**Tissue Processing**

The testis of both the experimental and control groups were removed and washed in normal saline. This organ was preserved in 10% buffered formalin to stop putrefaction. The tissue processing involves the following stages: dehydration, clearing, infiltration, embedding, sectioning (microtomy), staining and mounting. The processed tissues were then subjected to microscopy for histopathological analysis.

**RESULTS**

The transverse section of the testis of Wistar rats given water and feed showed well-structured interstitial connective tissues, interstitial Leydig cells, various stages of spermatogenic cells and seminiferous tubules with sperm cells (Figure 2).

The transverse section of the testis of the animals in group II given 3_mg/kg of cadmium only for 28_days showed interstitial connective tissue degeneration, detachment of the seminiferous tubules from their support, Leydig cells and spermatogenic cell necrosis and formation of solid masses of tumors within the lumen of the seminiferous tubules (Figure 3). Figure 4 showed the transverse section of the testis of the animals in group 3 given 3_mg/kg of cadmium for 28_days and 500_mg/kg of *Uvaria chamae* root extract for 28_days respectively, revealing rejuvenation of the interstitial connective tissue with persistent Leydig cell necrosis and continuous formation of solid masses of tumours within the lumen of the seminiferous tubules.

Figure 5 showed the transverse section of the testis of animals in group 4 given 3_mg/kg of cadmium for 28_days and 1000_mg/kg of *Uvaria chamae* root extract for 28_days revealing the rejuvenation of the interstitial connective tissue at several regions within the testis, presence of spermatogenic cells, disappearance and reduction of the solid masses of tumour in majority of the seminiferous tubules but with few tumour-like growth within few seminiferous tubules.

Figure 6 showed the transverse section of the testis of animals in group 5 given 3_mg/kg of cadmium for 28_days and 1500_mg/kg of *Uvaria chamae* root extract for 28_days revealing rejuvenation of the interstitial connective tissue at several regions within the testis, presence of spermatogenic cells and disappearance as well as reduction of the solid masses of tumour in majority of the seminiferous tubules.

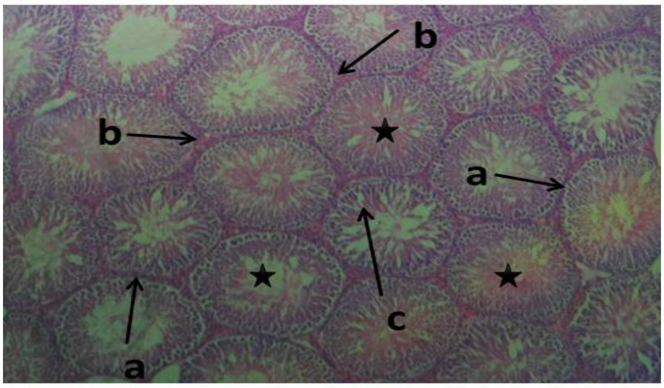


Figure 2: Photomicrograph of testis of control animals given water and feed alone showing, **a**- well-structured interstitial connective tissue, **b**-interstitial cells of Leydig, **c**- spermatogenic cells at various stages and intact seminiferous tubules with the presence of sperm cells (**stars**). H&E, 10x magnification.

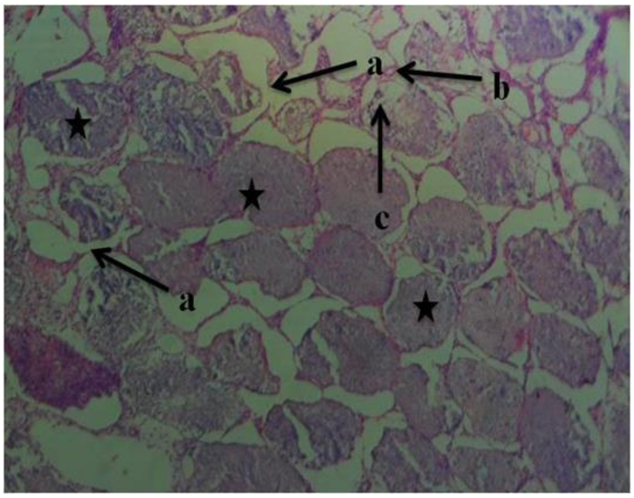


Figure 3: Photomicrograph of testis of group 2 animals given 3_mg/kg of cadmium alone for 28_days showing, **a**- degeneration of interstitial connective tissue and detachment of the seminiferous tubules from its support, **b**- Leydig cell necrosis, **c**- spermatogenic cells necrosis and formation of solid masses of tumour within the lumen of the seminiferous tubules (**stars**). H&E, 10x magnification.

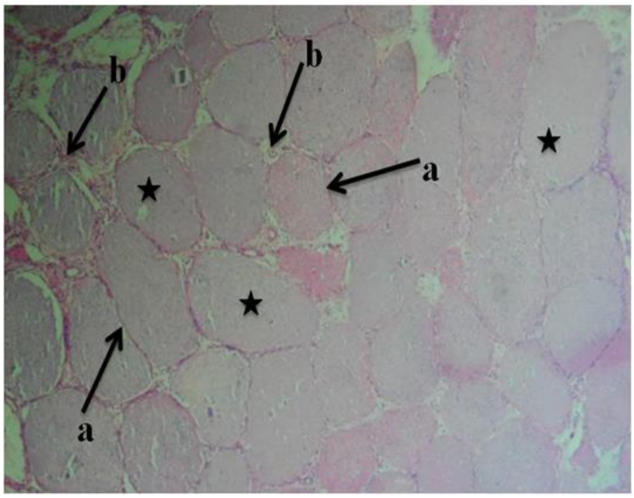


Figure 4: Photomicrograph of testis of group 3 animals given 3_mg/kg of cadmium for 28_days and 500_mg/kg of *Uvaria chamae* for 28_days showing, **a**- rejuvenation of the interstitial connective tissue at several regions within the testis but still with, **b**- the presence of Leydig cells necrosis and formation of solid masses of tumour within the lumen of the seminiferous tubules (**stars**). H&E, 10x magnification.

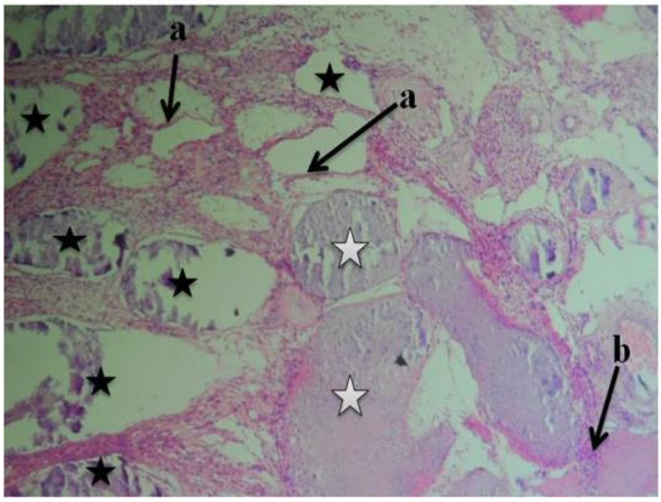


Figure 5: Photomicrograph of testis of group 4 animals given 3_mg/kg of cadmium for 28_days and 1000_mg/kg of *Uvaria chamae* for 28_days showing, **a**-rejuvenation of the interstitial connective tissue at several regions within the testis, **b**-presence of spermatogenic cells, disappearance and reduction of the solid masses of tumour in majority of the tubules (**black stars**) but with few tumor-like growth within few tubules (**white stars**). H&E, 10x magnification.

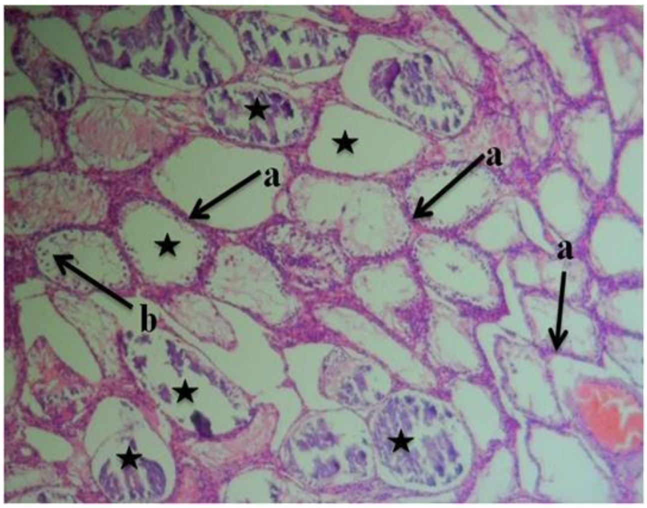


Figure 6: Photomicrograph of testis of group 5 animals given 3_mg/kg of cadmium for 28_days and 1500_mg/kg of *Uvaria chamae* for 28_days showing, **a**-rejuvenation of the interstitial connective tissue at several regions within the testis, **b**-spermatogenic cells and disappearance/ reduction of the solid masses of tumour in majority of the tubules (**stars**). H&E, 10x magnification.

**DISCUSSION**

From time immemorial, cadmium levels in the environment increased predominantly due to industrialization (OECD, 1996). In recent times, the use of cadmium in the industrial sector such as, the production of PVC products, color pigments, and Ni-Cd batteries has increased, thus causing a surge in cadmium exposure to the human population. According to Sharma *et al*. (2015) the bioaccumulation of cadmium in human the body and in the food chain leads to acute and chronic intoxications due to bio-magnification and this causes health effects which include diarrhea, stomach pains, bone fracture, reproductive failure and possibly infertility, damage to the central nervous system and immune system as well as psychological disorders. Cadmium can also cause the transformation of normal epithelial cells into carcinogenic cells by inhibiting the biosynthesis of protein. This essentially means that, cadmium can cause cancer which explains the presence of tumors in the animals that were induced with cadmium.

The results of this study revealed intriguing findings, offering valuable insights into the impact of *Uvaria chamae* root extract on the histo-morphology of the testes. The current study delves into the intricate relationship between cadmium exposure, a known reproductive toxin, and the potential ameliorative effects of *Uvaria chamae* root extract on testicular morphology, and the findings shed light on a spectrum of outcomes, ranging from severe impairment to pronounced ameliorative effects, thereby contributing to the existing body of knowledge on male fertility. The concept of phytotherapeutics in male infertility has garnered attention, with Camargo *et al*. (2018) highlighting the potential of various plant extracts in ameliorating the adverse effects of environmental toxins, and while the current study focuses on *Uvaria chamae* root extract, exploring its efficacy alongside other botanical extracts could provide a comprehensive understanding of the synergistic or antagonistic interactions between different herbal compounds.

A comprehensive synthesis of these studies could offer valuable insights into the multifaceted nature of herbal interventions in male reproductive health. Histo-morphological examinations of the testes provide a structural backdrop to the functional alterations observed in testicular histo-structure, and the stark contrast between the control group and the cadmium-exposed group illustrates the profound impact of cadmium on testicular architecture, as seen in the detachment of seminiferous tubules, Leydig cell necrosis, and the formation of tumor-like masses within the tubules which showed the disruptive effects of cadmium. *Uvaria chamae* root intervention, particularly at higher doses (Groups 4 and 5), unveils a restorative function as seen in the rejuvenation of interstitial connective tissue, presence of spermatogenic cells, and a reduction in tumor-like masses signifying the potential of *Uvaria chamae* root extract to not only preserve but also restore the structural integrity of the testes. These histological insights are in line with the findings of Godt *et al*. (2006), who demonstrated the reparative effects of herbal extracts on testicular tissues in a model of toxin-induced damage.

The results of the current study resonate with the broader scientific discourse on the detrimental effects of environmental toxins, particularly cadmium, on male reproductive parameters, and the observed significant formation of solid mass tumor in the testes in cadmium-exposed group is consistent with the findings of Pant *et al*. (2014), who reported similar outcomes in response to environmental toxin exposure. This shared observation underscores the detrimental impact of cadmium on testicular histo-structure, emphasizing the urgency for interventions to address these adverse effects.

The dose-dependent response observed in this study aligns with the results reported by Enabulele and Ifeka (2022), who explored the effects of herbal extracts on sperm concentration, and their findings, akin to the current study, suggested that higher doses of herbal extracts may exert a more pronounced positive impact on testicular histo-structure. Also, the work of Emordi *et al*. (2016) adds a layer of complexity by introducing herbal extracts as potential interventions, aligning with the current study's exploration of *Uvaria chamae* root extract. However, variations in methodologies, experimental designs, and dosage regimens across these studies necessitate a thorough interpretation of the comparative findings, and the intricacies of herbal medicine interactions with specific toxins and their varied effects on testicular histo-structure underscore the need for tailored approaches in addressing male infertility.

Arab *et al*. (2022) highlighted the anti-inflammatory properties of specific herbal compounds, which could potentially mitigate inflammation associated with toxin exposure, and inflammation in the testicular micro-environment is a known contributor to impaired spermatogenesis and decreased sperm concentration. The significance of these morphological assessments becomes particularly pronounced when considering the potential impact of *Uvaria chamae* root extract in the presence of cadmium-induced testicular solid mass tumor. The histo-morphological assessment of the control group unraveled an interplay of well-structured interstitial connective tissue, intact seminiferous tubules, and spermatogenic cells at various stages, and this portrayal of normal testicular architecture provides a baseline for comparison and underscores the intricate organization required for optimal reproductive function. These observations align with established literature (Arab *et al*., 2022), affirming the reliability of this study's histological methodology. Conversely, the cadmium-exposed group unveiled a distressing panorama of testicular degeneration. The degeneration of interstitial connective tissue, Leydig cell necrosis, and spermatogenic cell necrosis underscored the profound impact of cadmium on the delicate balance of testicular microenvironment, and the formation of tumor-like masses within seminiferous tubules further accentuated the severity of cadmium-induced testicular toxicity. This aligns with the findings of Lovaković, 2020, who reported similar histological alterations in response to cadmium exposure. The comprehensive histo-morphological alterations observed in the cadmium-exposed group resonate with the broader body of research on heavy metal-induced testicular damage (Godt *et al*., 2006). The presented evidence further underscores the urgency of identifying interventions capable of mitigating such detrimental effects, thus paving the way for the exploration of *Uvaria chamae* root's potential therapeutic role.

The introduction of *Uvaria chamae* root extract into the experiment, particularly in Group 4 and 5 administered with higher doses, yielded remarkable improvements in the histo-morphology, and the rejuvenation of interstitial connective tissue indicated a capacity for *Uvaria chamae* root extract to repair and regenerate the structural components of the testes. The persistence of spermatogenic cells and the reduction in tumor-like masses within the seminiferous tubules as seen in the results of this study highlighted the potential ameliorating and anti-tumorigenic effects of *Uvaria chamae*. These promising outcomes align with the findings of Aboul-Azm (1979), who explored the protective effects of a different herbal extract against testicular damage induced by toxic substances, and this parallels with results across diverse herbal interventions suggesting a commonality in the mechanisms by which these natural compounds exert their protective as well as ameliorating influence on testicular tissues.

Comparisons with studies conducted by Gupta *et al*. (2017) and Godt *et al.* (2006) fortify the current study's findings, and the observed ameliorative effects of *Uvaria chamae* root extract aligns with the reported benefits of herbal extracts in mitigating testicular damage induced by toxic substances. While Godt *et al*. (2006) focused on a different herbal extract, the common thread lies in the demonstrated ability of herbal interventions to counteract the adverse effects of toxic insults on testicular histo-morphology. Also, the dosage-dependent response observed in this study, with higher doses of *Uvaria chamae* root extract exhibiting more pronounced effects, resonates with Furuya *et al.* (2006). Their study on a distinct herbal remedy showcased dosage sensitivity in conferring protective effects against testicular damage, and this shared pattern suggests a pronounced relationship between dosage and therapeutic efficacy that transcends the specific herbal compound under investigation. The findings not only underscore the detrimental impact of cadmium on testicular architecture but also emphasize the remarkable reparative potential of *Uvaria chamae* root extract.

As the study aligns with previous research, it contributes to the cumulative knowledge surrounding herbal interventions and their role in preserving testicular integrity. The dosage-dependent response prompts further investigation into optimal dosages for maximal therapeutic benefits. Additionally, the observed parallels with studies involving different herbal extracts call for collaborative research to unravel shared mechanisms and potential synergies in combating testicular damage. Comparing the results of the current study with previous research provides a broader perspective on the efficacy of *Uvaria chamae* root extract. For instance, Pant *et al.* (2014) reported similar amelioating effects of another herbal extract in mitigating the toxic impact of heavy metals on sperm parameters, and the consistency in findings across different herbal extracts suggests a commonality in their mechanisms of action, possibly involving antioxidant and anti-inflammatory properties. However, it is essential to note that variations in study design, dosage, and administration methods can influence outcomes, and while the present study focused on the specific effects of *Uvaria chamae* root extract, future research could explore potential synergies between different herbal extracts or combination therapies.

## CONCLUSION

The study provides valuable insights into the anti-tumor effects of *Uvaria chamae* root extract against cadmium-induced testicular solid mass tumor. These findings suggest a potential therapeutic role for *Uvaria chamae* root extract in addressing male reproductive dysfunction associated with environmental toxin exposure.

The observed dose-dependent effects warrant further exploration to determine optimal dosage regimens for maximal therapeutic benefits, and the study contributes to the broader understanding of herbal interventions in male reproductive health and emphasizes the need for precision in dosage considerations. Additionally, the research aligns with existing literature on the adverse effects of cadmium on male fertility and underscores the importance of exploring natural compounds as potential remedies.

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