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Review Article

The road map to proper dental pulp experiments in animal models

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ABSTRACT

Background: Animal models are essential for the development and comparison of new dental materials. However, working on the dental pulp of animal models can be challenging.
Purpose: To provide guidance for conducting proper dental pulp experiments in animal models.
Materials and Methods: The author conducted a literature search on the Google Scholar database to find papers related to pulp capping and animal modeling in dentistry. Based on her own experience in animal research and the findings from the literature review, the author provides practical advice for new researchers in this field.
Results: Dogs and monkeys have the size and morphology of teeth that facilitate accessibility while operating in the dental pulp. However, the response of rat dental pulp has been found to be identical to that of humans. Rat maxillary molars are often used for dental pulp studies. Anesthesia using a combination of ketamine and xylazine provides a suitable time for pulp procedures in rats, but it's important to note that anesthetized rats may remain alert despite receiving the correct dose. Attempting to increase the dosage can result in the rat being unable to recover from anesthesia. To ensure safe and effective anesthesia, it is recommended to administer only one-third of the ketamine dose. Xylazine re-dosing should be avoided. Dental researchers in the Middle East and Africa may face unique challenges, but working on rats is challenging worldwide. In the future, virtual reality and simulation may offer alternatives for experimental animals.
Conclusion: Wister Albino is the more suitable animal model to be used in dental pulp experiments. The checklist and flow chart of PRAISE 2021 are mandatory for dental researchers experimenting with the dental pulp of animal models.

1. Introduction

Animal studies are performed where working on humans is of unknown risk. Using animal models in studying human anatomy and physiology referred to the sixth century BCE.[1] Animal models have contributed to the development of vaccines and antibiotics and the understanding of human diseases.[2] In the dental field, the development and comparison of new materials previously required the use of animal testing.[3–5] Dental pulp's reaction to materials has been studied in various animal models.

In 1879, Witzel suggested that the only way to gain knowledge about pulp healing was through animal research on dogs or sheep. However, he believed that such research was complex. In 1922, Rebel conducted the first animal research that examined direct pulp capping on cats and dogs.[6]

Many studies have used rats to investigate the reactions of dental pulp.[7] Most of researchers have used Wister Albino rats.[3,4,8–13] However, some researchers used Sprague Dawley rats.[14]

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Guinea pigs,[15] monkeys,[16] rabbits,[17] and also dogs[7] have been used by some researchers.

Research on dental pulp reactions in animal models is crucial for advancing our understanding of dentistry. However, such experiments are not commonly conducted in the Middle East and Africa due to the high costs, complex research work, and lack of expertise in this field. Therefore, it's essential for dental researchers to become more familiar with animal studies.

This paper presents a roadmap for dental researchers who plan to conduct pulp experiments in animal models and outlines the potential challenges researchers may encounter during this type of experiment.

2. Materials and Methods

A search on literature was performed in the Google Scholar database using the keywords "Animal model," "Rat," "Wister Albino," "Direct," "Pulp capping," "Dental pulp," and "Molar teeth." The search selected only the papers published in English that demonstrated pulp capping performed in rats or studies that reviewed animal models in dentistry.

A snowballing method was performed by screening the reference list of the selected articles to choose relevant studies, papers describing the biology and tooth morphology of rats, and papers describing the protocol of anesthesia in research animals.

The author combined her scientific experience in animal projects with a literature review to offer helpful advice for new researchers interested in conducting dental pulp experiments using animal models.

2.1. Statistical analysis

During the search using the Google Scholar engine, 246 papers were found. Out of these, 68% were excluded based on their titles. The exclusion was for different reasons; 53% investigated parameters other than that of pulp capping like anti-inflammatory, antioxidant effects, trauma, and microbiological studies, 7% investigated the impact of materials on rats by subcutaneous testing, 6% were studies performed in rats but not related to dental pulp, 1.4% were clinical trials, and 1.4% were about tissue engineering.

After the first screening, the remaining 32% of the papers were passed through a second screening process, which involved removing duplicates and reading the abstracts. This led to a final selection of papers used in this review.

3. Result

3.1. Rat as an animal model for dental pulp experiment

Although monkeys are more closely related to humans in terms of evolution than any other animal, the dental pulp tissue reaction of monkeys is different than humans.[7]

According to standard 7405 of the International Organization for Standardization (ISO), only mammals such as monkeys, dogs, ferrets, or miniature pigs are suitable for animal research to assess the biocompatibility of dental materials when they come in direct contact with pulp tissue.[7,18] Rats have been used in 70 published studies over the past 50 years to assess dental pulp reaction despite not being explicitly mentioned as appropriate animals. Studies have shown that when calcium hydroxide is used in direct pulp capping for rats, the histological response of their dental pulp is comparable to that of humans. Additionally, other research has found that the response of rat dental pulp is identical to that of human dental pulp.[7] Many studies have found that direct pulp capping performed in rat molars is better comparable with humans than the results obtained from dogs.[7] After all these studies, scientists have found the rejection of experimental studies of pulp tissue in rat molar is unjustified.[7]

Despite numerous pulp experiments conducted on rat molars, some researchers found mice molars perfect for obtaining reliable data about pulp tissue reaction after direct pulp capping.[19]

3.2. Rat teeth[20]

In pulp experiments, rats are the most suitable subjects. Therefore, it is important to have a good understanding of the rat jaw anatomy. Rats are classified as monodonts because they have only one set of teeth. It consists of sixteen teeth, eight on each jaw. They do not have canines or premolars, and their jaw only consists of front incisors and posterior molars on each side. A long space called diastema separates the incisors from the molars on each side. (Figure 1 A,B)

Rat incisors are unique open-rooted teeth that are constantly worn down by gnawing. To balance this process, they undergo continuous growth. Within 8-10 days after birth, the incisors begin to emerge from the gum, and it takes about 40-50 days for them to reach the occlusal level.

Molars are used for grinding food and are located at the back of the mouth with three on each side of both jaws. The first molar erupts on the 19th day after birth, the second on the 21^{st} day, and the third molar at 35-40th day. Complete set of teeth are apparent by age of 6 weeks.

3.3. Morphology of the crown of the first maxillary molar

Many studies concerning pulp reactions towards different stimuli have been performed in the first maxillary molar.[3–5,21–23] It is the preferred tooth to be involved in dental pulp experiments on rats. Why?

In the anatomy of a rat's jaw (as seen in Figure 1A/B), the molar teeth are located towards the back due to the toothless gap between the incisors and molars. This positioning

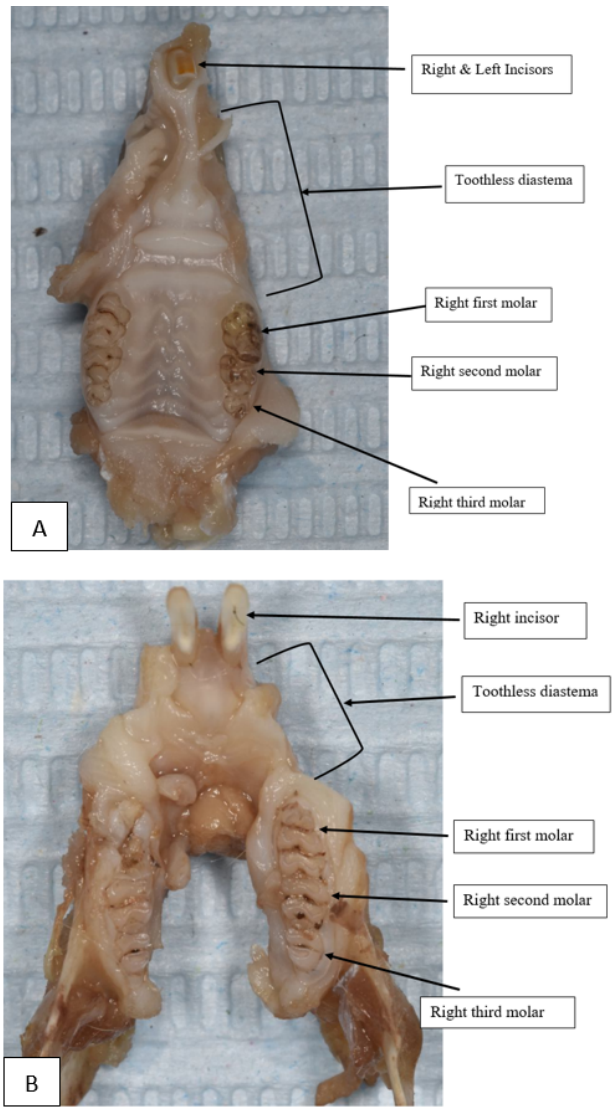

Figure 1: A): Upper jaw of Wister Albino Rat with its different parts; **B)**: Lower jaw of Wister Albino Rat with its different parts

makes the second and third molars difficult to reach most of the time. The mandibular molars are obstructed by the tongue and cheek, making them even harder to access. The rat's incisors are continuously worn down throughout its life, which makes it unsuitable for pulp capping experiments. However, some researchers have studied the reaction of pulp tissue to materials in incisors.[9,13] Excluding these limitations, the maxillary first molar is the most suitable option.

Maxillary first molar contains nine tubercles (T1- T9) occlusally. These tubercles are arranged in three crosswise rows. Each row contains three tubercles and is separated from the next row by a transverse groove. (Figure 2 A,B)

The first row of tubercles (T1, T2, and T3) is the most anterior, with T1 on the lingual side of the molar, T2 median,

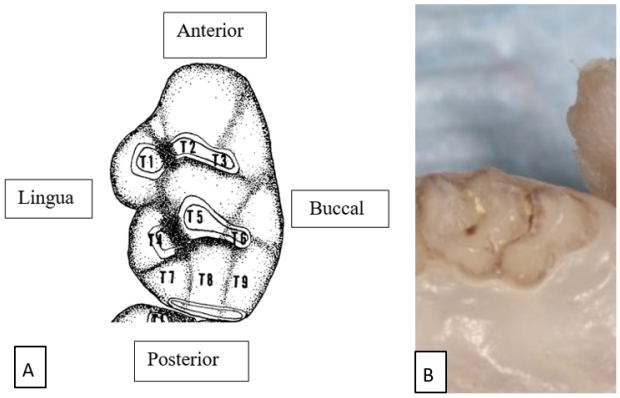

Figure 2: A): Occlusal view of first maxillary molar of caries-susceptible male of 25 days old. In courtesy of (Hunt, Rosen et al. 1970);[24] **B**): Occlusal view of first maxillary molar of 8 weeks age healthy male Wister Albino rat

and T3 on the buccal side. The second transverse row comprises T4 (lingual), T5 (median), and T6 (buccal). T7, T8, and T9 form the third row, a single broad plate with faint grooves indicating the markings between the tubercles.[24]

3.4. Considerations before starting dental pulp experiment in an animal model

After conducting animal experiments, the next step is the clinical translation of the findings to humans. However, this translation requires proper reporting of the animal study. Clear guidelines have been developed for reporting animal experiments in endodontics to ensure appropriate reporting. This set of guidelines is called Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021, which aims to improve the quality of animal experiments, methodology, and reproducibility. The guidelines consist of a checklist of 11 domains divided into 43 items, including the title, keywords, abstract, introduction, method, results, and discussion. Ten of these items pertain to methodology and involve reporting ethical approval, sample size, details of the animal model such as species, age, and weight, information of the intervention, medication or instrumentation, details on how animal suffering was prevented, the post-operative care of the animals, and the software and statistical analysis of the data.[25]

For a researcher planning to conduct dental pulp experiments on animals, it's recommended to refer to the PRAISE 2021 checklist after writing the research proposal and before starting the experiment. Going through this checklist will help ensure that the methodology is robust and will also bring to attention all ethical aspects that need to be considered during the study.

Many obstacles may face researchers in the Middle East and Africa while experimenting with the dental pulp of

Wister Albino rats.

The author encountered obstacles when working on Wister Albino rat's dental pulp studies. However, before discussing these obstacles, it is essential to highlight the significance of reporting the age and weight of the Wistar Albino models used in these studies. The age of the rat reflects its ability to regenerate pulp tissue, while its weight is crucial in determining the correct dosage of anesthesia.

The literature has reported that the weight of the Wistar Albino rat model ranged from 300-400 grams for 8-9 week-old rats,[5] 180-220 grams for 8-week-old rats,[3,4,11,21] and 150 grams for 6-7 week old rats.[23] Some studies that investigated dental pulp reactions only reported the weight of the Wistar Albino rat, which ranged from 200-250 grams,[10] while others mentioned only the age, which ranged from 6-8 weeks.[22] Based on the literature, the weight of an 8-week-old Wister Albino rat ranges from 180-400g.

3.4.1. The first obstacle

Based on the author's experience, the size of Wister Albino rats has a significant impact on experiments, with smaller rats presenting more challenges. The author faced a specific challenge related to rat weight, as the sizes of the 64 Wister Albino rats collected were smaller than what was reported in the literature. Among these, 17 rats were aged between 9-10 weeks, while the remaining 47 were aged between 7-8 weeks.

The group of 9-10 week-old Wister rats displayed a weight range of 70-99 grams, with an average weight of 83 grams. In comparison, the group of 7-8 week-old rats weighed between 53 to 96 grams, with an average weight of 77 grams. The observed variation in weight between what was reported in the literature and what was observed by the author (Figure 3) may reflect a genetic discrepancy in Wister Albino rats.

Figure 3: Small sized healthy male Wister Albino rats of 7-8 weeks age

3.5. What are the challenges of small-sized Wister rats?

Smaller Wister Albino rats have smaller mouth openings and teeth, making accessing the operative field difficult and requiring specialized small instruments. Accessing the dental pulp in such smaller Wisters may require tilting the handpiece of the long axis to penetrate the pulp diagonally instead of parallel to the long axis. This presents a dilemma for the researcher, who must choose between tilting the handpiece and compromising the standardization of the study or maintaining the proper position of the handpiece and opening the Wister's mouth wider than its capacity, which may result in tearing the Wister's mouth corners (Figure 4).

Figure 4: Tearing of mouth corner in small sized 8 weeks aged Wister Albino rat in an attempt to work with hand piece in first maxillary molar

3.5.1. The second obstacle

The anatomy of the Wister Albino rat's jaw presents a unique challenge. The jaw contains a long gap between the incisors and molars, without any teeth (Figure 1 A). The upper first molar, which is the preferred tooth for pulp experiments, is located far back due to this long gap. This position makes it difficult to access the upper first molar, particularly in small-sized rats. Additionally, the visibility of the posterior molar teeth is hindered by the tongue and cheeks.

3.5.2. The third obstacle

The housing of rats is controlled by scientific guidelines.[26] As a result, only a limited number of rat houses are available. Due to the growing interest in research across various medical fields, there is an increased demand for

Wister Albino rats among researchers and postgraduate students. However, the limited housing availability and the high demand for Wister Albino rats have made it difficult to obtain them during certain seasons, causing challenges for researchers.

3.5.3. The fourth obstacle

When conducting dental pulp experiments using Wister rats, it is often necessary to determine their age. However, in other medical fields such as veterinary science, pharmacy, and medicine, researchers are more interested in the weight of the Wister rats rather than the age. As a result, not all rat houses keep records of the age of their rats. This makes it difficult for dental researchers who require rats of a specific age, as they must monitor newborn rats until they reach the desired age before starting the experiment.[22]

3.5.4. The fifth obstacle

Conducting experiments on Wister Albino rats, especially in dentistry, is expensive for the researcher. This is primarily due to the costs of housing the rats throughout the experiment. These expenses include the cost of purchasing the rats, the cost of providing them with food throughout the experiment, as well as the cost of hiring someone to care for them. The longer the experiment duration, the higher the costs will be. Dental pulp experiments on Wister rats usually run for 14 days,[3,4,22,23] 28 days[3–5,13,22,23] or 30 days[11] which is relatively long period. Therefore, it is recommended that such experiments are funded appropriately.

3.5.5. The sixth obstacle

Handling rats requires specialized courses, which may not be available in some countries of the Middle East and Africa. Accordingly, the experiment can take longer than scheduled.

3.5.6. The seventh obstacle

Obtaining the proper position of the rat to access its dental field needs a particular unit or operating board. It should be designed before enrollment in the experiment (Figure 5). The operator should be situated behind the rat while it lies on its back (Figures 6 and 7). Working without a specific dental unit for the rat may compromise its airway, leading to the rat's death (Figure 8).

4. Discussion

Dogs and monkeys have the size and morphology of teeth that facilitate accessibility and visibility during operative work in the dental pulp. In contrast, rats have challenging small size teeth. Despite all these facts, rats have been recommended as a practical model in dental pulp experiments because rats are easy to be handled and have good resistance to infection,[27] as well as a faster metabolism

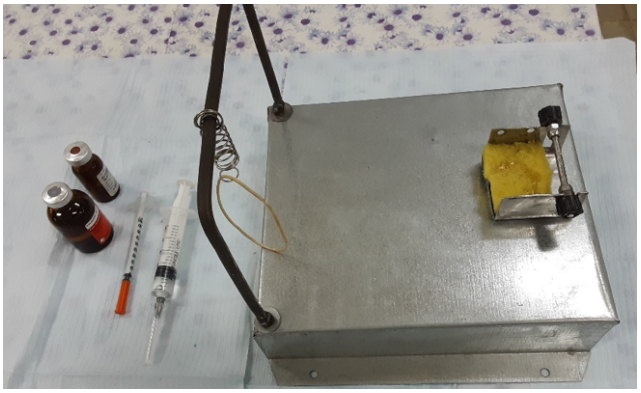

Figure 5: Dental unit designed to obtain appropriate position of rat during operative work

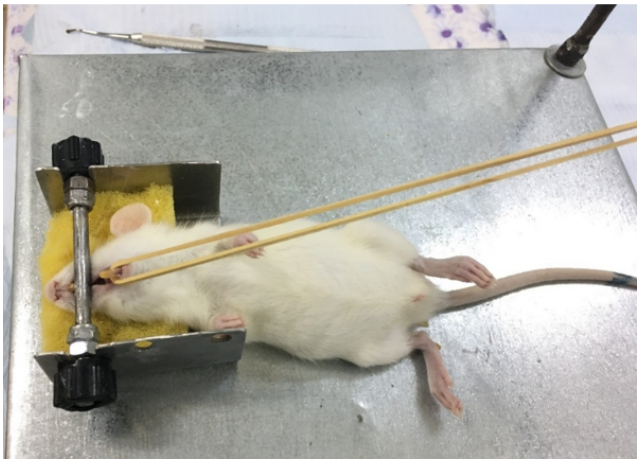

Figure 6: Wister Albino rat laid down the dental unit ready for operative work

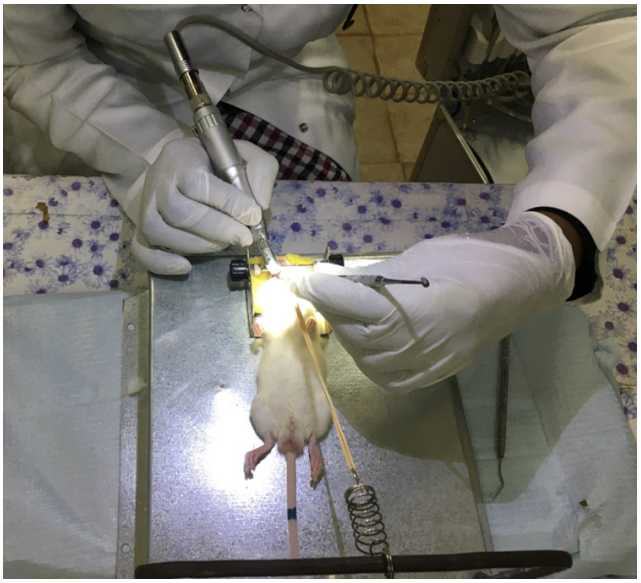

Figure 7: The appropriate operator position to work on first maxillary molar on Wister Albino

Figure 8: Dental work without operating board or special rat dental unit compromising the airway by fingers and may endanger the tongue in an attempt to retract it

of rats which reduces the time of research.[7,28] Moreover, rat maxillary molars have been used to simulate the human molar teeth for direct pulp capping due to their similarity in anatomical, biological, and histological features, as well as the pulpal repair process.[7]

This paper addresses the challenges that dental researchers in the Middle East and Africa may face, which may not be true for dental researchers in other regions. However, even for other researchers, working on the Wistar Albino dental pulp can be challenging.

When working with rats, unexpected deaths may occur during or after work. Anesthesia is an essential factor to be considered to reduce unexpected death. A combination of ketamine and xylazine is the preferred injectable anesthetic for rats. It can be injected intraperitoneal or subcutaneously to produce 45-90 minutes of anesthesia.[29]

Based on the literature,[29,30] an easy and practical method for preparing a suitable dose of anesthesia for Wistar albino rats has been proposed by the author:

Step 1: Ketamine 50mg/ml and xylazine 20mg/ml is mixed with distilled water which acts as a carrier according to the following formula:

Ketamine (4ml) +xylazine (1ml) + Distilled water (1ml) = (6ml) of prepared anesthesia (anesthetic mixture).

Step 2: The dose of anesthesia is calculated and collected from the anesthetic mixture prepared on step 1 according to the following equation:

Dose (ml) = 0.23× Rat weight (grams) /100

Based on the author's experience, the intraperitoneal injection of this anesthetic mixture typically lasts between 30 and 40 minutes for Wister albino rats. However, some rats may remain alert despite receiving the correct dose. Attempting to increase the dosage can result in the rat being unable to recover from anesthesia. The sensitivity of rats to different anesthetics can vary significantly depending on factors such as age, body composition, strain, health status, genetic manipulation, and sex. To ensure safe and effective anesthesia in case of redosing, the Penn State Animal Resource Programme recommends using only one-third of the dose of ketamine and avoiding redosing of xylazine unless absolutely necessary.[30]

Animal studies have provided invaluable information, but they are costly and have some limitations. Nevertheless, they are mandatory before the clinical implications of new materials and drugs. Modern advancement in medical field would not have been made without preclinical animal modelling.

Recently, there has been an increased focus on animal welfare, with growing concerns about the unnecessary use of animals in research. The use of animals in experiments should be minimized. Virtual reality and simulation offer alternatives for experimental animals in the future.[18] Till that time, researchers who work with animal models must be extremely mindful of the ethical considerations surrounding their use.

5. Conclusion

Wister Albino is the more suitable animal model to be used in dental pulp experiments. The checklist and flow chart of PRAISE 2021 are mandatory for dental researchers experimenting with the dental pulp of animal models. It is crucial for the researcher to know the anatomy of rat teeth before starting the experiments. Special settings, equipment, and experts in this field are necessary to minimize unexpected events.

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7. Author Contribution

Nuha Elmubarak, the correspondence author, is responsible for writing the original draft, review, editing and finalization of the manuscript.

8. Data Availability Statement

All the data used during the current study are available within the manuscript.

9. Conflicts of Interest

The author declares no Conflicts of interest.

10. Ethical Consideration

The author has performed her animal project in accordance with the internationally accepted principles for laboratory animal use and care. It has been approved by Sudan veterinary council.

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