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Original Research Article

Convenient and efficient method for ingestion of L-NAME to induce hypertension in rats: An alternative to oral gavage

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Abstract

Objectives: Administration of drug by oral route is used since a long time in Indian traditional medicine and experimental procedures. Oral gavage is one of the best methods to deliver drug to animals, but it need skilled hand with various harmful effects. So an alternative to oral gavage is warranted now a day.

Materials and Methods: The study was conducted on twelve male wistar rats randomly divided into two groups: Group A (n=6) and Group B (n=6). L-NAME was given orally to animals of group B for 21 days daily in the morning time in a dosage of 40mg/kg body weight to induce hypertension. L-NAME was mixed in special custom made food (vehicle) which was highly acceptable and easily consumed by rats which deliver L-NAME in full required dose. Body weight, systolic blood pressure and heart rate were recorded and analyzed before and after giving L-NAME.

Results: Systolic blood pressure was increased from 120.95 ± 4.81 mmHg to 133.74 ± 5.90 mmHg and heart rate was significantly reduced from 305.12 ± 10.25 bpm to 290.11 ± 11.23 bpm after administration of L-NAME for 21 days.

Conclusion: This new alternative method of oral gavage is highly effective and sensitive as we successfully induce hypertension in rats without any harm or mortality in animals.

Keywords: L-NAME, Hypertension, Oral Gavage.

1. Introduction

Administration of drug by oral route is used since a long time in Indian traditional medicine as well as in modern medicine due to convenient and physiologically accepted method [1]. To increase absorption and effectiveness of drug parenteral routes including intravenous and subcutaneous are also in practice [2]. For experimental studies, in which animals are subjected to voluntary ingestion of solid drugs, are given by oral route is a very difficult task. Gastric feeding by gastric tube is also used in many studies but this method may leads to injury to gut wall in animal [3]. Oral gavage is one of the best methods to deliver drug to animals, but it has been observed that this method need skilled hand with various precautions because little mistakes can be harmful to animal. Studies indicate that drugs can be administered orally by adding them to food or in liquid diet [4]. For proper administration it is essential that drug should be intermingled with some food stuff which would be acceptable and edible to animals. The disadvantages of such type of strategy are the variation in the dose to be received by each animal and unpleasant taste of drug.

Some drugs are bitter in taste therefore oral gavage is not easily acceptable by animals. In our experimental induction of hypertension was done by L-NAME (40mg/kg body weight) along with food [5]. For the administration of precise dose of drug it is essential that food mixed with drug must be fully consumed. Therefore we introduced a novel mixture of food which is edible, preferential to animal choice, non toxic and does not contain any substance which could affect normal metabolism of animal.

2. Material and Method

The study was conducted in Department of Physiology, King George's Medical University, Lucknow and study protocol was approved by Institutional ethical committee (Letter no.41/IAH/Pharma-17). A total of 12 male wistar rats were procured from IITR Lucknow. Animals were housed in central animal house of the University. Utmost care was taken to avoid any harm during the stay of animals. Animals were kept in 12 hour day night cycle at the room temperature of 25-28° C.

Animals were divided into control and experimental group consisting of 6 animals each. During the process of ingestion of drug the animals were kept in separate cages to avoid counter eating of food.

2.1 Preparation of Special vehicle (food mixture):

Vehicle was prepared by mixing roasted gram flour (8gm), roasted wheat flour (2gm), roasted corn flour (2gm), sugar (1gm) and Indian Desi Ghee (3gm). All above ingredients were roasted in Desi Ghee till it starts giving nutty aroma. It requires a lot of hand work to stir the mixture till a proper texture is obtained. It is important to roast the flour well till starts releasing ghee and a proper texture is obtained.

2.2 Induction of hypertension

Experimental group (n=6):

The drug L-NAME in dosage 40mg/kg body weight mixed with custom made special food (3gm) was given to animals of experimental group after 12 hr fasting at 9 AM every day for 21 days. The standard rat chow was given in prescribed doses [6] for rest of the day and water was given *ad libitum.*

Control group (n=6):

The animals of control group were treated with freshly prepared custom made special food (3gm) (without L-NAME) after 12 hour fasting at 9AM every day. The standard rat chow in prescribed doses was given for rest of the day and water was given *ad libitum.* This protocol was followed for 21 days.

2.2.1 Measurement of animal body weight

The weight of each animal was recorded in grams with standard weighing machine at 10AM weekly during whole of study period in both groups.

2.2.2 Measurement of Systolic Blood Pressure (SBP) and Heart Rate (HR)

Blood Pressure and Heart rate were measured by Non Invasive Blood Pressure (NIBP) monitoring machine every week during the whole study period in both control and experimental group of animals. SBP and HR were measured by methods used by earlier studies [7-9].

In brief, after acclimatizing animals they were kept in restrainers, the tail was passed through the hole of the cuff and the pulse transducer was tied around the tail distal to the NIBP cuff. SBP and HR were measured by NIBP automatically.

3. Observation and results

Baseline body weight (gm) of animals of control group was 234.17 ± 5.51 while the body weight of experimental animals was 237.67 ± 6.18. We observed no significant difference between control and experimental group. Baseline SBP (mmHg) of animals of control group was 118.42 ± 8.20 while the body weight of experimental animals was 117.94 ± 6.45. It was observed that there was no significant difference in SBP between control and experimental group. There was no significant difference in baseline reading of HR (beats/min). It was 304.83 ± 7.56 in control group and 311.72 ± 9.61 in experimental group.

After 1st and 2nd week of ingestion of L-NAME and Placebo, we observed that there was no significant difference in Body weight, SBP and HR. **(Table 1)**

After completion of protocol of 21 days we found that the mean body weight in control group was 236.33 ± 4.69 while in experimental group it was 216.66 ± 5.41, which was significantly decreased (p=0.001). SBP was 120.85 ± 4.81 in control group while it was133.74 ± 5.90 in experimental group of animals showing a highly significant difference (p=0.002). HR was found 305.12 ± 10.25 in control group and 290.11 ± 11.23 in experimental group which was decreased significantly (p=0.036). **(Table 1)**

Table 1: Effect of administration of L-NAME along with custom made vehicle

Parameters during protocol / Groups	Control (n=6)	Experimental (n=6)	'p' value
Weight (gm)			
Baseline	234.17±5.51	237.67±6.18	0.324
After 1 week	234.38±4.57	235.24±5.96	0.784
After 2 week	233.41±3.12	230.12±5.33	0.221
After 3 week	236.33±4.69	216.66±5.41	0.001
Systolic BP (mmHg)			
Baseline	118.42 ±8.20	117.94 ± 6.45	0.912
After 1 week	116.43 ±7.35	117.76± 5.32	0.711
After 2 week	120.68 ± 6.45	124.84± 7.82	0.339
After 3week	120.95 ± 4.81	133.74 ± 5.90	0.002
Heart Rate (bpm)			
Baseline	304.83±7.56	311.72±9.61	0.197
After 1 week	310.22±6.20	312.29±5.26	0.546
After 2 week	308.62±10.65	305.18±9.64	0.570
After 3week	305.12±10.25	290.11±11.23	0.036

Bpm: beats per minute;
't' test applied between control vs experimental groups

4. Discussion

In conscious experimental animals like rat, oral route is most convenient for administration of any drug or chemical substances. Oral gavage technique is traditionally used in laboratories by experimental scientist across the world [4]. Gavage is the procedure in which the substance is introduced into the stomach by means of a tube. In this technique, it is necessary to train the animal for a period of one week during which the rats learned to drink a vehicle solution [3]. Skilled technician is absolute necessary for

giving oral gavage. This technique is highly effective, but care must be taken to avoid complications including the damage of oral cavity, esophagus and aspiration of substance through trachea. High mortality rate (32%) due to asphyxia and injuries in oropharynx was observed by various authors [10]. Oral gavage also attributed to cause stress in animals during the procedure [11]. Various modifications in gavage techniques were introduced to reduce morbidity and mortality in laboratory animals including flexible cannulas but found ineffective to minimize complications [12]. Therefore an alternative convenient and efficient method is in need of the day in experimental laboratories. In present study we aimed to replace oral gavage with easy and effective method for oral ingestion of drug.

In our study we prepared a special type of vehicle which is edible and preferable to animals. This food (vehicle) was immediately consumed by the animals without any wastage so that the drug which was mixed in the vehicle was fully delivered. The fragrance and taste of vehicle attracted the animals so that the animals consumed it very fast without any training. The ingredients used in the preparation of vehicle were easily available and no training was required. Our data showed that L-NAME administration along with custom made special food (vehicle) successfully induces hypertension. Hence the above said vehicle has advantages over oral gavage method. Limitation of this study was that the animals need 12 hr overnight fasting which may have any psychological or physiological effects on animals.

5. Conclusion

We successfully induced hypertension in experimental animals by a newer technique of oral administration of drug. The specialized custom made food was preferably eaten by all animals in prescribed dosage without any specialized training to animals. This technique has advantage over the traditional oral gavages in which mortality and morbidity was associated. Thus the current technique can be incorporated in place of oral gavage.

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