UNIVERSITI PUTRA MALAYSIA

EFFECT OF HERBAL POWDER, *Ferula asafoetida,* ON THE CONCEPTION RATE OF RATS

UMIKA KANHYE

EFFECT OF HERBAL POWDER, *Ferula asafoetida,* ON THE CONCEPTION RATE OF RATS

UMIKA KANHYE

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

MARCH 2016

CERTIFICATION

It is hereby certified that I have read this project paper entitled "Effect of Herbal Powder, *Ferula asafoetida,* on the Conception Rate of Rats", by Umika Kanhye and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 - Project.

DR. HAFANDI BIN AHMAD

DVM (UPM), M.Sc (UPM), Ph.D (La Trobe University, Australia)

Senior lecturer

Department of Veterinary Pre Clinical Science

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

DEDICATIONS

I dedicate this project to my beloved Mom, Dad and sister who have encouraged and supported me morally through my five year course and my final year project.

ACKNOWLEDGEMENTS

To the persons that have assisted me throughout this project, I would firstly like to thank my supervisor, Dr Hafandi Bin Ahmad for the guidance, expertise and time that he granted me throughout the duration of my project, and my studies at the faculty.

A special thanks also goes to Dr Murugaiyah Marimuthu for giving me such an interesting project title and being by my side to willingly share his knowledge to help.

I would also like to thank Dr Farina Mustaffa and animal house UPM staff En. Kamarand En. Noraziman for their patience and help.

A special thanks to all my classmate of DVM 2016 who assisted me directly or indirectly in this project with special mention to Santhini Bhaskaran.

Lastly, endless gratitude to my family who were always by my side through the ups and downs. They always cheered me up and never gave up on me.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek

KESAN SERBUK HERBA, *Ferula asafoetida*, PADA KADAR KEHAMILAN TIKUS

Oleh

Umika Kanhye

2016

Penyelia: Dr. Hafandi Ahmad

Penyelia bersama:

Prof. Madya. Dr. Shaik Mohd Amin

Dr. Murugaiyah Marimuthu

Tikus merupakan vector utama dari leptospira dan pelbagai lagi penyakit bawaan vektor. Menurut laporan dari Persatuan Kawalan Haiwan Perosak di Malaysia, populasi tikus di Kuala Lumpur pada tahun 2010 adalah 4.4 juta dan tahun 2013 sebanyak 6.8 juta. Walaupun jumlah tangkapan dilakukan secara besar-besaran, populasi vector ini tidak berkurangan dan sebab utama ialah kapasiti pembiakan tikus yang sangat pesat. Salah satu cara untuk mengawal pembiakan populasi tikus ini adalah dengan menggunakan herba, *Ferula asafoetida*, yang mempunyai ciri kontraseptif. Oleh itu, kajian ini bertujuan untuk melihat kesan serbuk *Ferula asafoetida* pada kadar pembiakan tikus. Seekor jantan dan enam ekor betina tikus jenis Sprague dawley telah digunakan untuk kajian ini. Tiga ekor betina telah diberikan 400 mg serbuk *asafoetida*

dicampur dalam air setiap hari, manakala tiga ekor dari kumpulan kawalan diberi air normal. Haiwan jantan kemudiannya disatukan ke dalam sangkar haiwan betina untuk mengawan. Keputusan menunjukkan peningkatan pesat dalam berat badan, buncit abdomen dan rambut sekitar puting pada tikus kumpulan kawalan. Bagi kumpulan rawatan, tidak ada tanda kehamilan diperhatikan; menjurus kepada dua faktor yang mungkin iaitu *pheromone (bauan)* haiwan betina atau kegagalan implantasi. Kesimpulannya, serbuk *asafoetida* adalah semulajadi, herba yang selamat untuk mengawal populasi tikus dan penyebaran penyakit.

Kata kunci: populasi tikus, *Ferula asafoetida,* kawalan biologi

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999- Project

EFFECT OF HERBAL POWDER, *Ferula asafoetida*, ON THE CONCEPTION RATE OF RATS

By

Umika Kanhye

2016

Supervisor: Dr. Hafandi Ahmad

Co-supervisors:

Assoc. Prof. Dr. Shaik Mohd Amin

Dr. Murugaiyah Marimuthu

Rats are reservoirs for Leptospirosis and many vector borne diseases. According to the Malaysian Pest Control Association, the rat population in Kuala Lumpur, in 2010, was 4.4 million and 2013, 6.8 million. Despite massive trapping, the population did not decrease significantly and the main reason being the rapid rat breeding capacity. One of the ways to control breeding is to use a local herb, *Ferula asafoetida*, which has contraceptive properties. This study aimed to observe the effect of *asafoetida* powder on the conception rate of rats. One male and six female Sprague dawley rats were used for this study. Three female were given 400 mg of *asafoetida* powder mixed in water daily as a treatment group, while the other three from the control group were given fresh water. The male was introduced to the female cage for one week mating. Results showed that animals in control group increased their body weight, had distended

abdomen and hair loss around the teats. However, for the treatment group there was no pregnancy sign observed; leading to two possible factors, masking of female pheromone or failure of implantation. Thus, this could indicate that *asafoetida* powder is a natural, safe herb to control rat population and the spread of diseases.

Key words: rat population, *Ferula asafoetida*, biological control

1.0 INTRODUCTION

1.1 Rats (*Rattus* sp.)

Rats are thin long tailed, medium-sized rodents of the family Muridae. They are originated from Asia and Australia but are now found all over world. The best known rat species to humans are the black rats, *Rattus rattus*, and the brown rats, *Rattus norvegicus.* Both black rats and brown rats are omnivorous consuming nearly anything digestible, especially store grains. However, brown rat prefers carnivorous diet, aggressively pursuing a wide variety of prey including shrimp, snails, mussels, insects, bird eggs and young, amphibians, eels, fish, pheasant, pigeons, poultry, rabbits, and carrion. Rats find shelter around pipes, behind walls, near garbage cans, in deep rock crevices and in dwellings from small village huts to large city buildings (Musser, 2014).

1.2 Rat population in Malaysia

According to the Malaysian Pest Control Association, the estimated rat population in metropolitan Kuala Lumpur, in 2010, was estimated at 4.4 million and in 2013, 6.8 million rats (Kuala Lumpur City Hall report, 2013). The common sightings of rodents in eateries, residential areas, markets, recreational parks and many other places in broad daylight in the cities (Kuala Lumpur, Johor Bahru, Georgetown) is a clear indication that the rat population has increased many fold. According to an estimate by a local renown rodent biologist there are eight rodents to one person in the country (Liat, 2013)

1.3 Effects on human

A scientific literature search on rodent diseases revealed that rodents are reservoirs for about 70 different types of diseases spread to humans worldwide. These diseases are spread to human directly due to a bite wound by a disease carrying rodent or indirectly via consuming food or water that have been contaminated with rodent feces and urine.

Rats have been identified as the main cause of leptospirosis in livestock and human according to a study published by the Malaysian Society of Parasitology and Tropical Medicine. In 2012, there were 3,665 cases of leptospirosis reported by the Health Ministry's disease control division. Whilst in 2010, there were 1,976 cases recorded with 69 deaths and in 2011, 2,268 cases with 55 deaths. For 2013, during the increase in rat population the leptospirosis cases also increased by almost two times with a number of 4457 cases and 71 deaths (Ministry of Health Malaysia, 2013). Foreign experts said that the control of the rat population is critical in preventing outbreak of leptospirosis (Bahaman 2000)

1.4 Control of rat population

The two most common control methods are poisoning and hunting and trapping. For poisoning, various bait mixtures were used in early time and this included sodium arsenite, thallium sulphate and zinc phosphide. However, behavioural resistance (bait avoidance) was observed few years later after the implementation of this control technique. This implied that the rats refused to take the usually attractive baits in lethal quantity. This behaviour appeared to be heritable leading to the failure of this technique (Greaves, 1994). Nonetheless, at present new bait mixtures are prepared and given in food materials like rhinoceros beetle (Oryctes rhinoceros) grubs and many more.

The second technique, hunting and trapping, showed some success in reducing the rat population. However, with an increasing population, more resources were needed to effectively control the population size. The Municipal Council of Penang Island (MPPP) reported that 14,639 rats were caught and killed in 2009. In 2013, with a population of 6.8 million, poison and traps were no longer effective as control methods.

1.5 Biological control

An early approach to control rat population, beside bait poisoning, was the introduction of Barn owl (*Tyto alba*). In one study by Smal in 1990, 99.4 % of owl's diet was found to be rats. This approach showed a decrease in the population, however with a shortage of nesting sites limited the growth of barn owl populations (Duckett 1991, in Smith 1994). Study done by Smal (1990) reported that one breeding pair per 6 to 8 ha was required for owls to effectively control rat populations. Therefore the introduction of nest boxes (one per 5 ha) increased the owl density to the requisite level and it was the aggregation and dispersal of non-breeding juvenile owls that enabled the predator to respond to fluctuations in rat densities.

2.0 LITERATURE REVIEW

2.1 *Ferula asafoetida*

Figure 1: *Ferula asafetida* plant, extracted oleo-gum resin and powder form

Ferula asafetida, a herbal perennial plant, comes from the umbelliferae family, commonly known as the celery, carrot or parsley family. It is known as 'inggu' in Malay, 'perungaayam' in Tamil and 'Hing' in Hindi. It grows wild from Eastern Mediterranean to Central Asia at elevations of 0.61 to 1.22 km on plains that are arid in winter and where few other plants survive. The plant reaches a height of up to 2 m and bears clusters of pale, greenish-yellow flowers and an oval fruit. Interestingly, all parts of the plant have a distinctive fetid odor (Mahendra, 2012).

The oleo gum-resin, called asafetida, is extracted from the roots and rhizomes of the plant. Upon incisions on the roots, a milky liquid oozes out which then dries to form a resin in brown colour. A single plant may yield up to 1 kg of resin before the plant dries out (Rossetti, 2009). Asafetida can be found in Indian shop in Malaysia in the form of a solid brick or even smaller pieces, along with tablet or powder form known

as ‘compounded asafoetida’**.** It is mainly added in indian dishes such as curry and gravy, in a small amount (1 pinch), to add flavor to the dish.

2.1.1 Properties and traditional uses

Traditionally, asafoetida powder is used for the treatment of various diseases, such as asthma, epilepsy, stomach-ache, flatulence, intestinal parasites, weak digestion and influenza in human (Bisht, 2012). In human, it is taken for problems associated with impotency for man, while in women it reduces menstrual pain and also acts as a contraceptive agent towards unwanted pregnancy (Mak, 2014). The herbal powder is consumed mostly by Indians community for their dishes. In fact, recent studies showed that asafoetida possess several activities such as antioxidant, antiviral, antifungal, cancer chemopreventive, antidiabetic, antispasmodic, hypotensive and molluscicidal (Mahendra et al., 2012).

2.1.2 Composition of *Ferula asafoetida*

Asafetida consists of 4% to 20% volatile oil, 40% to 60% resin and 25% gum (Eigner, 1999). The essential oil (20%) contains a wealth of sulfur compounds, mainly disulfides as its major components such (R)-2-butyl-1-propenyl disulphide (50%), 1-(1-methylthiopropyl) 1-propenyl disulphide and 2-butyl-3-methylthioallyl disulphide. Furthermore, di-2-butyl trisulphide, 2-butyl methyl trisulphide, di-2-butyl disulphide and even di-2-butyl tetrasulphide have been found. The organic sulphur containing compounds give the putrid odor and bitter, acrid taste. The essential oil also contains

some terpenes (α-pinene, phellandrenes) and hendecylsulphonyl acetic acid. The resin contains ferulic acid, umbelliferone, asaresinotannols, farnesiferols A, B, and C and kamalonol. The gum is composed of glucose, galactose, arabinose, rhamnose and glucuronic acid (Mahendra et al., 2012).

Table 1: The pharmacological activities of asafetida with their respective chemical constituents causing it

Pharmacological activity	**Responsible chemical constituent**
Anticancer	α-pinene; α-terpineol; diallyl-disulphide; ferulic acid; isopimpinellin; luteolin; umbelliferone; vanilin
Anti-inflammatory	α-pinene; azulene; β-pinene; ferulic acid; isopimpinelin; luteolin; umbelliferone
Antiviral	α-pinene; diallyl-disulphide; ferulic acid; luteolin; vanillin
Antibacterial	α-pinene; α-terpineol; azulene; diallyl-disulphide; diallyl-sulfide; ferulic acid; luteolin; umbelliferone
Antioxidant	Ferulic acid; luteolin; vanillin
Anti-prostaglandin	Umbelliferone

2.2 Effect of another herbal plant, *Gendarussa vulgaris,* **on the reproductive system of rats**

This herbal plant originates from India, growing in beds of streams in moisture areas of the Himalayas. It has properties such as antispasmodic, antiperiodic, analgesic, anti-inflammatory, anti-flatulence, emetic, anti-pyretic and laxative. However, there are few studies reporting that this herbal plant causes damage to the reproductive system leading to infertility. According to a study done by Wan (2015), this Indian herbal plant, when fed to mice, caused irreversible damage to male and female reproductive systems, liver as well as kidneys.

3.0 MATERIALS AND METHODS

3.1 Animals

Seven Sprague dawley rats, including one male with good fertility and 6 females who conceived before were kept at 24 ^{o}C in plastic cages. The male rat was kept in one cage while the six females were separated in two groups of three rats each; the control group and the treatment group. The experimental design was such that the male cage was located in between the female cages to induce estrus in the females (Whitten, 1998).

The cages had a stainless steel mesh cover and saw dust as bedding material. Fresh water and food pellets were given *ad libitum* for all groups during the first 4 days of acclimatisation. The initial weights of the rats were noted to be around 200-250 g and were monitored on a daily basis. The amount of feed intake was also monitored daily.

After 4 days of acclimatisation, the treatment regime was started for the treatment group while the control group received fresh water and pellets. The male was then introduced to the control group for 4 hours for mating, after which he was transferred to the treatment group for mating as well. The reception of the male to the females and vice versa as well as mating behaviour was observed and recorded.

3.3 Treatment

The treatment plan was started after 4 days of acclimatisation. The amount, of *asafoetida* powder (400 mg/kg; 3 g) were measured and mixed in 125 mL of fresh water. The powder dissolved after continuous vigorous stirring to give a light brown solution. This treatment water was given to the treatment group daily for 21 days, and each time fresh water and herbal powder were used.

3.4 General observation

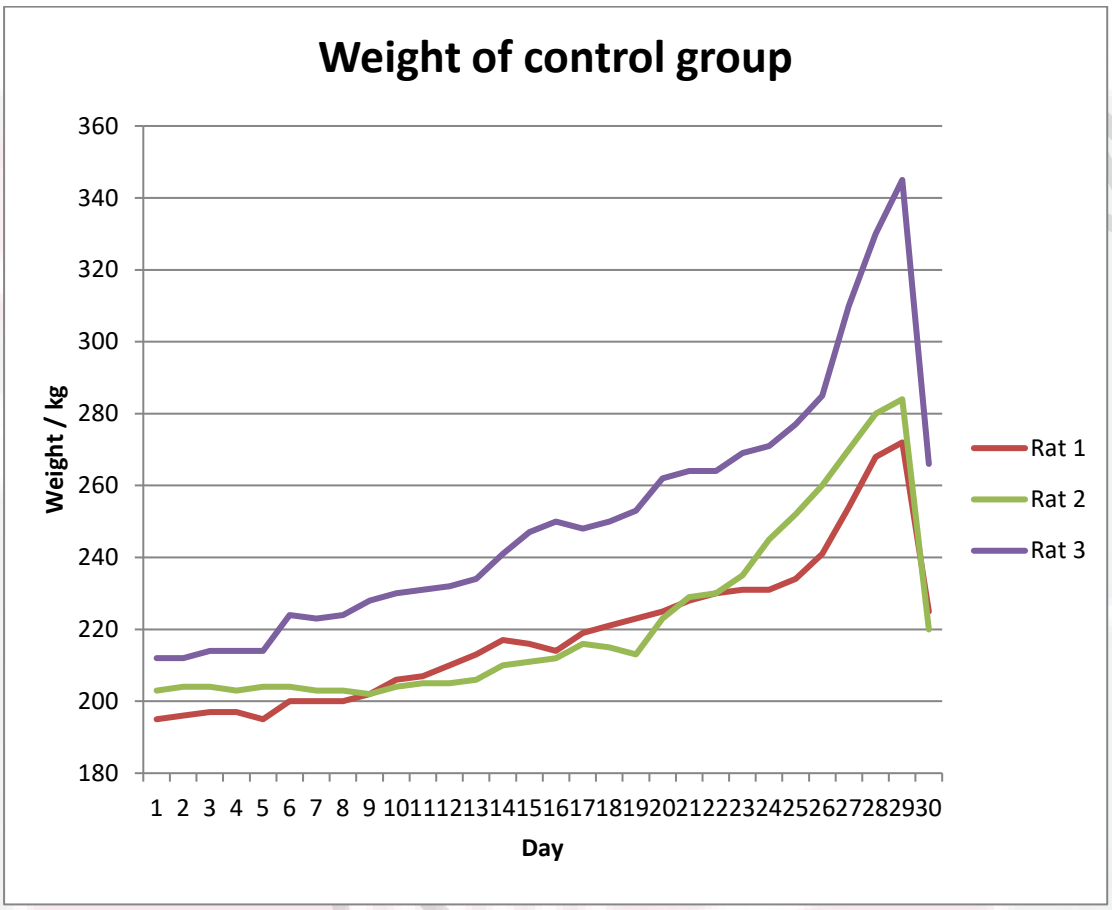
Any physical changes such as distended abdomen, appearance of prominent pinkish teats, hair loss around the teats were monitored.

4.0 RESULTS

4.1 Weight of rats

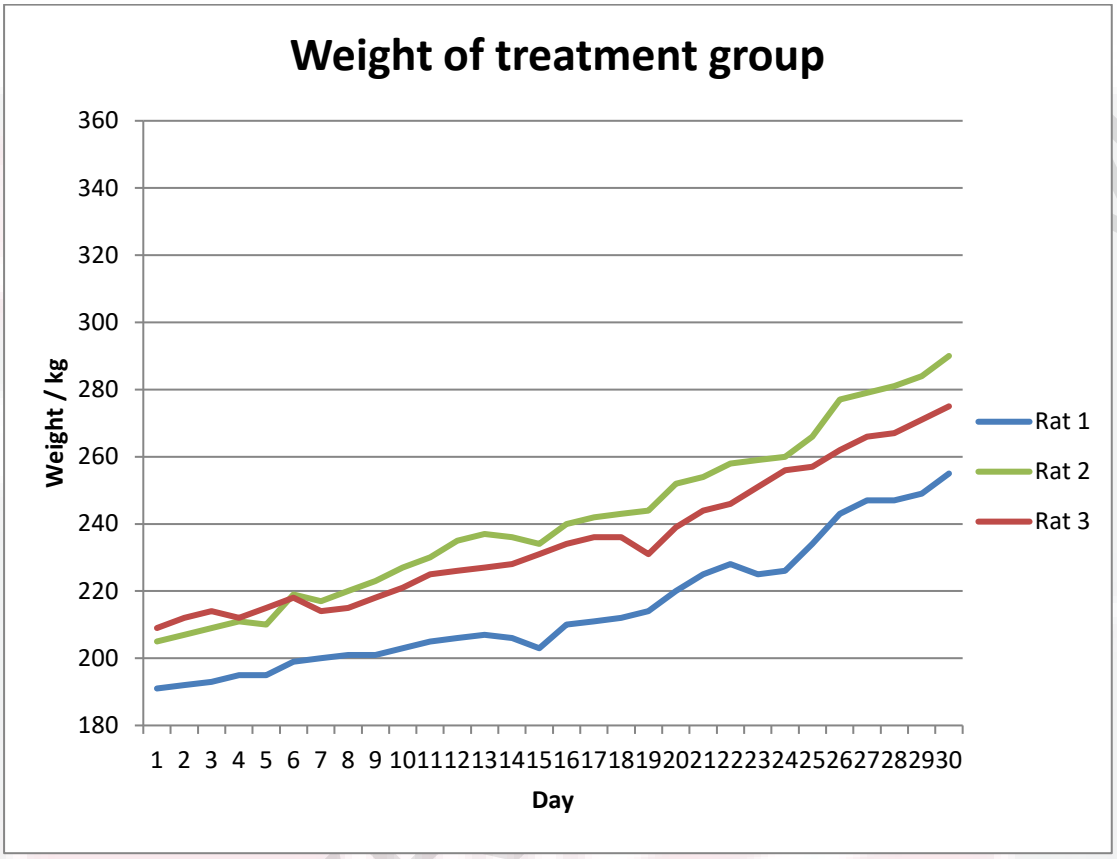
The body weight of control female rats 1, 2 and 3 were measured on day 1 and recorded at 195g, 203g and 212g respectively. These animals had a gradual increased the body weight of 1-2g daily from day 1 to day 5. On day 6, an abrupt large increase in the body weight was observed in these rats. Rats 1, 2, 3 gained 5, 4 and 10 g each respectively within 24 hours. This was suggestive of positive pregnancy in all 3 rats. From day 6 onwards, the body weight measured daily had an increasing gradient and reached its maximum peak on day 29 with a body weight of 272g, 284g and 345g for rats 1, 2 and 3, respectively. This confirmed that the control rats were pregnant. On day 30 there was a drop in body weight measured in all rats whereby the body weights recorded were 225g, 220g and 266g, respectively. Therefore on day 29 the female rats delivered their pups after which the body weights measured on the next day showed a lower value. Figure 2 shows the body weight chart of the control group from day 1 to day 30.

Figure 2: Daily body weight gain chart for the female rats in control group



For the treatment group, the body weight of rats 1, 2 and 3 were measured at 191g, 205g and 209g, respectively. During the course of 30 days there was a gradual constant increased in body weight of 2-3g per day. At the end of 30 days, though the male was introduced to the female cage for mating, there was no large increase in body weight. The maximum weight gain for rat 1, 2 and 3 was on day 30 with values of 255g, 290g and 275g, respectively. As compared to the body weight chart of the control group, it can be presumed that the treatment group rats were not pregnant due to different graph pattern of their body weight. This is illustrated in Figure 3 as shown below.

Figure 3: Daily body weight gain chart for the female rats in treatment group

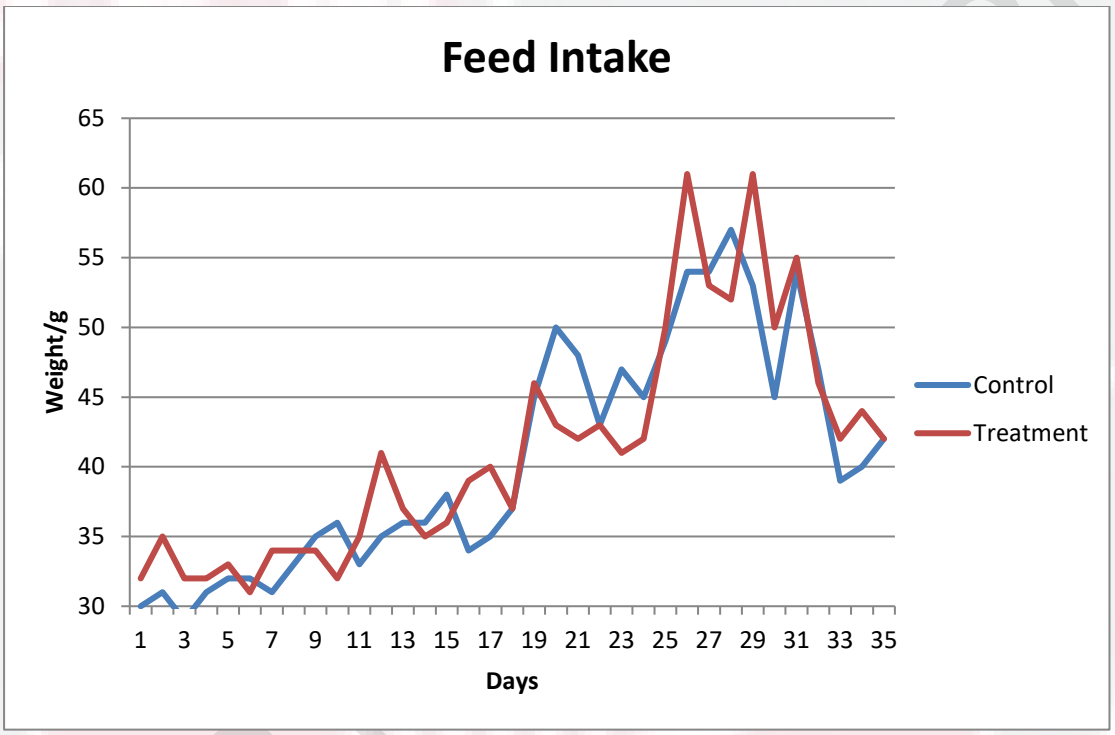


4.2 Feed intake

The daily feed intake of the female rats in both groups was measured and recorded. Both groups had an increase in feed intake from day 1 to day 30. The normal feed intake in rats is 5g of feed per 100g of body weight. Therefore for an average weight of 203g per rat the feed intake per day should be 10g of feed. With a total of 3 rats per group the total feed intake per cage should be 30g. However for both the control group and treatment group the feed intake was higher than that with a maximum intake of 60g of feed on day 25 onwards. Nevertheless, the treatment group had a slightly higher value as compared to the control group. This could indicate that the weight gain

in the treatment group was due to the higher feed intake and not due to pregnancy. The feed intake chart is illustrated in Figure 4 below.

Figure 4: Daily feed intake chart for both control and treatment groups



4.3 General observation

The prominent pregnancy signs for the control animals were shown on day 25 onwards whereby all the 3 control rats had distended abdomen. The teats were pink in colour and enlarged. There was also hair loss around the teats as shown in Figure 5. On day 29, nesting behaviour, such as shredding tissue paper given into smaller sizes and lining it in the corner of the cage, was observed. On the same day each female delivered 10 pups in average as shown in Figure 6 below.

For the treatment group, there were no pregnancy related physical changes to the body from day 1 to day 30. Figure 7 below shows the picture of one of the female rat from the treatment group on day 25 with no prominent teats or distended abdomen.

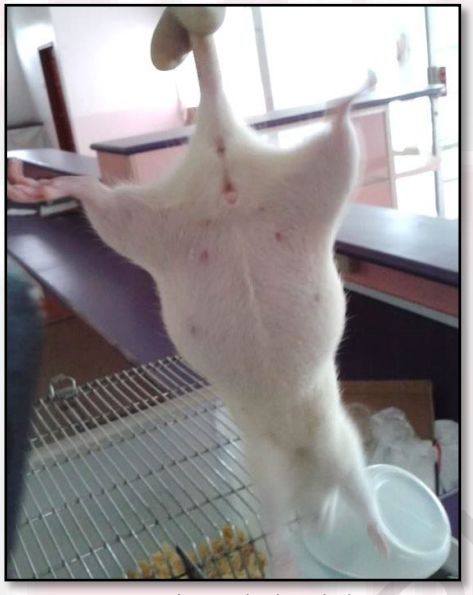


Figure 5: Distended abdomen and prominent pink teats with hair loss around teats of control rat on Day 25

Figure 6: Delivery of pups on Day 29 with an average of 10 pups per control rat

Figure 7: No pregnancy signs or physical changes in treatment rat

5.0 DISCUSSION

According to an article published by Karen in 2005, a female rat may begin to gain weight within 24 hours after mating. A daily increase gives assurance throughout the pregnancy that babies are growing within. The amount of weight the female gains depends on her body mass at onset of the pregnancy, her diet, as well as the number of pups she is carrying. A very clear pattern of immediate significant weight gain followed by a period of slow steady gain with a very significant weight gain the last few days can be observed. A typical fetus at full term averages 5-6 grams. At term females with a decent sized litter can gain as much as 90 grams.

The treatment group was confirmed to be non-pregnant of animals. However, there are two main factors that could have led to this consequence. Firstly, there could be pheromone masking of the female and secondly the impairment in the implantation process. According to Wickes in (1998) who reported that the fetid odour of asafoetida is imparted to the breath, secretions, flatus, and gastric eructation. Therefore, this could have masked the pheromones of the female rats of the treatment group. After the introduction of the male rat to the female cage, with the low or no pheromone level of the female, the male was not stimulated for mating. Thus, no mating occurred resulting in no pregnancy. However, this depends on the volume of treatment water intake. If there is less treatment water intake and still no pregnancy noted then the second factor comes into play.

In 2004, there was a study done by Bajpai which states that plants lacking phytoestrogens may intercept pregnancy by their ability to disrupt energy metabolism in rat uterus during implantation. Therefore, based on this study *Ferula asafoetida* plant

has been proven to lack phytoestrogens which is a naturally occurring plant nutrient that exerts an estrogen-like action on the body. This implies that if there is successful mating followed by successful fertilisation of the ovum then there is possibility of pregnancy. However, since the rats were given this herbal plant that lacks phytoestrogen, therefore there is impairment in implantation due to low energy level.

6.0 CONCLUSION

Ferula asafoetida, herbal powder, reduces the conception rate in rat so that it is an efficient biological control of rat population. By this natural technique we can control the spread of diseases such as leptospirosis, salmonellosis and other vector borne diseases. Moreover, it is a safe and non-toxic product to the environment since it is a natural product consumed by many people.

7.0 RECOMMENDATION

Firstly, different dosage of the asafoetida powder can be given to different groups of female rats to know the minimum dosage needed to produce significant effects on the conception rate. Secondly, the effect of the herbal powder on the fertility of the male can be studied.

8.0 REFERENCES

Agosta, W.G. (1992). Chemical communication: the language of pheromones. *Scientific American Library, New York.*

Battersby, S.A., Parsons, R. & Webster, J.P. (2002). Urban rat infestations and the risk to public health. *Journal of Environmental Health Research,* 1(2).

Bradford, A. (2015). Facts about Rats. *Live science contributor.*

Corrigan, R. M. (2011). Rats and mice. In Mallis, A., Moreland, D. & Hedges, S.A. *The Mallis Handbook of Pest Control.* Cleveland: GIE Publications, 10,11–119.

Debbie. (2013). Rat Reproduction. *The rat fan club.*

Donnelly, M.T. (2015). Overview of rodents: *The Merck Veterinary Manual*.

Fong, L.F., Tan. S. & Raman, A. (2013). City Hall: 6.8 million rats in KL. *The star news Malaysia.*

Hahn, D., Ericson, W., & Probst, A., (1981). Antifertility activity of *Montanoa tomentosa*n: Contraception. *Indian Journal Pharmacology*, 23, 133-140.

Katzer, G. (2003). *Ferula asafoetida* origin. Retrieved from Spices Pages http://gernot-katzers-spice-pages.com/engl/Feru_ass.html on 10th January 2016.

Keshri, G., Bajpai, M., Lakshmi, V., Setty, B.S. & Gupta, G. (2004). Role of energy metabolism in the pregnancy interceptive action of Ferula assafoetida and Melia azedarach extracts in rat. *PubMed,* 70(5), 429-432.

Keshri, G., Bajpai, M., Lakshmi, V., Setty, S. & Gupta, G. (2004). Role of energy metabolism in the pregnancy interceptive action of *Ferula asafoetida* and *Melia azedarach* extracts in rat: Contraception. *Indian Journal Pharmacology*, 70(5), 429-432.

Lai, I., Wong, P.M., Henry, E., Zieman. (2013). Rat posing a serious problem in Malaysia. *The star news.*

Londonkar, R., & Nayaka, B. (2011, October). Evaluation of the anti-implantation and abortifacient properties of *Portulaca oleracea* in abino rats. *International Journal of Pharma and Bo sciences,* 2 (4), 501-508.

Mahendra, P., & Bisht, S. (2012, December 6). *Ferula asafoetida*: Traditional uses and pharmacological activities. *Pharmacognosy Review*, *12*, 141-146.

Ministry of Health Malaysia. (2010). Estimated 4.4 million rats in KL, *Press statement.*

Misra, M., & Misra, R.(1999). Screening of a few indigenous abortifacients. *Journal of the Indian Medical Association,* 525-535.

Musser, G. (2014). Rat: Rodent genus. *Encyclopaedia Britannica.*

Nurhakimah, W. (2015). The effect of *Gendarussa vulgaris* methanolic extract on the reproductive organs of female mice. UPM, Malaysia.

Param, S. (2012). Ecto and Endo Parasites of Malaysian Rodents - Human and Veterinary Implications. *Lambert Academic Publishing Co German*, pp. 148.

Param, S. (2013). Growing rat population a serious health threat. *Free Malaysia today.*

Rizuan, M.A., Hisham N.H., Hafidzi, M.N. & Sukri, T.I. (2014). Bio-control of rats: The birds of prey along the shore of the Felda oil palm plantation areas, Borneo Malaysia. *The proceeding of the 8th International Conference on Plant Protection in the Tropics (ICPPT).*

Smith, K. (2004). Examination of Vaginal smears in the rat: The Laboratory Animal Unit. *Norwegian school of Veterinary science.*

Suckow, M., Weisbroth, S. (). The laboratory rat: Reproduction and Breeding. *American college of Laboratory,* 2, 145-152.

Tamboo, L. (2013). 6.8 million rats make KL their home. *Astroawani News Malaysia.*

Tan, C., Foong, J. (2010, November 26). Rodent threat. *The star news.*

Tan, C., Foong, J. (2011, February). Rat menace in Klang valley. *The star newsdesk,* pp. 3.

Theagarajan, V. (2012). Health Benefits of *asafoetida.*

Westwood, R. (2008). The Female Rat Reproductive Cycle: A Practical Histological Guide to Staging. *Sage Journal,* 36 (3), 375-384.

Wickes, H., Lloyd, J. (1998). *Ferula asafoetida,* Henriette's Herbal Homepage. *King's American Dispensatory.*

Wood, B.J. & Chung, G.F. (2002). A critical review of the development of rat control in Malaysian agriculture since the 1960s. *Merrivale Research,* 22, 445-461.

Zainal, D. (2014). KL markets slammed for being a rat's paradise. Rat population in 2013.

Zainudin, A.W. (2015). Epidemiology and current situation of Leptospirosis in Malaysia. Ministry of Health Malaysia.