

THE EFFECT OF BEEBREAD ON WOUND HEALING IN
MALNOURISHED RABBITS

By

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ABBREVIATION

Kruskal-Walis Test	KWT
Standar deviation	SD
Interquatile Range	IQR
United State Surgical Corporation	USSC

ABSTRACT

Background

Beebread is a honey bee product whose completeness in the range of all essential food components is well documented in many published data (Mercola, J. 2003) .It is made up of bee pollen mixed with enzymes secreted by honey bees The use of Bee Pollen as a Super food has been documented by Liebelt, R.A., Calcaginetti D.(1999).: The effects of a bee pollen diet in promoting growth of laboratory rat has been demonstrated (*American Bee Journal*,**139**(5): 390-395).It contains the whole range of protein, essential amino acids and fatty acids, carbohydrates as well as vitamins and minerals including trace elements ,(Campos, M.G., R.F. Webby and K.R. Markham 2002). The unique occurrence of the flavone aglycone tricetin in bee pollen has been sited by Campos(M. G 2002) .It contains flavonoids, Selenium in high quantity as well as other active anti-oxidant elements, and antibacterial effect (Baltrusaityte 2007) V. P.R. Venskutonis and V. Ceksteryte, demonstrated the radical scavenging activity of honey and beebread phenolic extracts, *Food Chem.*, **101**: 502-514.

Aim of the current study

Despite the above mentioned qualities of beebread, there is not much of literature documenting relevant scientific research with regard to its application in clinical medicine. The aim of this study is to explore the potentials of its nutritional value in improving post operative wound healing in malnourished rabbits.

Methodology

A set of 30 white New Zealand rabbits were used for the study.Six were used for base line data while 24 were subjected to malnutrition by being fed with 50% of their

daily food requirement for 30 days and then subjected to surgery (laparotomy). They were then randomly divided into two groups of 12 rabbits each. The control group on normal rabbit diet and supplemented group received 30% beebread supplementation in their diet. Half of the supplemented group (6 rabbits out of 12) and half from the control group (6 rabbits out of 12) were sacrificed on day 7 post laparotomy. Tissues from the laparotomy wound of the sacrificed rabbit were harvested and subjected to a analysis for tensile strength, fibroblast content, neovascularization, epithelization and hydroxyproline content as assessment tools for quality of wound healing.

Another set of 6 rabbits each from the supplemented group and control group were sacrificed on day 14 after surgery the same analysis as the first set.

Results

Data obtained from the above analysis indicated significant difference between the control group on normal rabbit diet and the supplemented group receiving 30% of their daily diet as beebread supplementation with a p-value < 0.05 with regard to tensile strength, fibroblasts and neovascularization. The result for the epithelization and hydroxyproline did not indicate a significant difference with p-value > 0.05 .

Conclusion

The result of the study showed better wound healing parameters such as wound tensile strength, neovascularization and fibroblast count in the incision wound of the supplemented group of rabbits but there was no significant difference in the epithelization and hydroxyproline content of the supplemented group compared to the control group. This experiment therefore indicates the possibility of the use of beebread in malnourished patients to improve surgical outcome.

ABSTRAK

Background

Beebread adalah produk madu lebah yang kesempurnaan dalam lingkungan semua komponen makanan penting didokumenkan dengan baik dalam banyak data published (Mercola, J. 2003). It terdiri daripada debunga lebah dicampur dengan enzim yang dirembeskan oleh lebah madu Penggunaan Bee Pollen sebagai makanan super yang telah dilaporkan oleh Liebelt, RA, Calcagineti D. (1999) .: kesan diet debunga lebah dalam menggalakkan pertumbuhan tikus makmal telah demonstrated (American Journal Bee, 139 (5): 390-39 Tujuan kajian semasa

Walaupun ciri-ciri yang disebutkan di atas daripada beebread, tidak banyak kesusasteraan mendokumenkan penyelidikan saintifik yang berkaitan berkenaan dengan permohonan dalam bidang perubatan klinikal. Tujuan kajian ini adalah untuk meneroka potensi nilai pemakanan dalam meningkatkan penyembuhan luka selepas pembedahan dalam arnab kekurangan zat makanan.

Metodologi

Satu set 30 putih arnab New Zealand telah digunakan untuk study. Six digunakan untuk data base talian manakala 24 tertakluk kepada kekurangan zat makanan dengan diberi makan dengan 50% daripada mereka keperluan harian makanan selama 30 hari dan kemudian tertakluk kepada pembedahan (laparotomy). Mereka kemudian dibahagikan secara rawak kepada dua kumpulan 12 setiap arnab. Kumpulan kawalan pada diet arnab normal dan kumpulan ditambah menerima 30% suplemen beebread dalam diet. Half mereka daripada kumpulan yang ditambah (6 arnab daripada 12) dan separuh daripada kumpulan kawalan (6 arnab daripada 12) dikorbankan pada hari 7 pos laparotomy . Tisu dari luka laparotomy arnab berkorban dituai dan subjeted kepada analisis untuk kekuatan tegangan, kandungan fibroblast, neovascularization, epitelization dan kandungan hydroxyproline sebagai alat penilaian untuk kualiti penyembuhan luka. Satu lagi set 6 arnab setiap daripada kumpulan kawalan dan kumpulan supplementd telah sacrificed pada hari ke-14 selepas pembedahan analisis yang sama dengan set pertama.

Keputusan

Data yang diperolehi daripada analisis di atas, terdapat perbezaan yang signifikan antara kumpulan kawalan pada diet arnab normal dan kumpulan supplemented menerima 30% daripada diet harian mereka sebagai suplemen beebread dengan nilai- $p < 0.05$ dengan mengambil kira strength tegangan, fibroblas dan hasil

neovascularization. The untuk epithelization dan hydroxyproline tidak menunjukkan difference signifikan dengan p-nilai > 0.05.

Kesimpulan

Hasil kajian menunjukkan luka yang lebih baik penyembuhan parameter seperti kekuatan tegangan luka, neovascularization dan fibroblast hitungan luka insisi kumpulan the supplemented arnab tetapi tidak ada difference ketara dalam epithelization dan kandungan hydroxyproline kumpulan ditambah berbanding dengan kumpulan kawalan eksperimen. This itu menunjukkan possibility penggunaan beebread pada pesakit yang kekurangan zat makanan untuk meningkatkan pembedahan keluar datang.

5) .Ia mengandungi keseluruhan julat protein, asid amino dan asid lemak, karbohidrat serta vitamin dan mineral termasuk kandungan (, Campos, MG, RF Webby dan KR Markham 2002). Kejadian unik tricetin flavone aglycone dalam debunga lebah telah ditempatkan oleh Campos (M. G 2002) .Ia mengandungi flavonoid, Selenium dalam kuantiti yang tinggi serta lain-lain unsur-unsur anti-oksida aktif, dan kesan anti-bakteria (Baltrusaityte 2007) VPR Venskutonis dan V. Ceksteryte, menunjukkan aktiviti memerangkap radikal madu dan ekstrak fenolik beebread, Makanan Chem, 101.: 502-514.

INTRODUCTION

TITLE: THE EFFECT OF BEEBREAD SUPPLEMENTATION ON WOUND HEALING IN MALNOURISHED RABBITS

Bismillahir-Rahmanir - Rahim

“And your Lord inspired the bees saying: “Take your habitations in the mountains and in the trees and in what they (people) erect. Then eat of all fruits, and follow the ways of your Lord made easy (for you).” There comes forth from their bellies, a drink of varying colours (and types) wherein is a healing for mankind. Verily, in this indeed is a sign for people who are thoughtful.”

(CHAPTER OF THE BEE- AL- QURAN) (1400 years ago)

Verses 68-69

1.1 Background of the study

Beebread is an amorphous brownish substance made by worker bee from pollen grains, nectar and honey. It is used for feeding the young bees. To make it more digestible for the young bees, it is mixed with digestive enzymes and inoculated with useful non-pathogenic bacteria for fermentation. In this form it can be stored in the bee comb for up

to five years without losing its nutritional value,(Herbert and Shimanuki, 1978; Alfonsus, 1933;)

Chemical Composition of Beebread

The chemical composition of beebread varies from region to region and also from season to season depending on the floral content of that region and the seasonal variation. The content of a beebread of standard nutritional value irrespective of region contains all the essential components as described below.

- Beebread is a highly nutritious compound containing all the essential food elements
- Protein mainly in the form of amino acids – 25 – 30%
- Carbohydrates in the form of simple sugars-30-55%
- Essential fatty acids-1.5 – 20%
- High amount of vitamins (full range of vitamins; A,B,C,D,E, Folic acid)
- Essential trace minerals and elements including selenium(highest amount in natural biologic product) Flavonoids-which have antibacterial, anti inflammatory and antioxidant activity(Stanciu et al 2009, Serra Bonvehi et al 2001, Moreira et al 2008)



Fig 1: Bees storing beebread in its hive (Adapted from google images)



Fig : 2 Bee collecting nectar (for honey) and pollen (for beebread) (adapted from google image)



Fig 3: Pure beebread (adapted from Google Image)



Fig 4 :Beebread Nuggets(adapted from google Image)



Fig:5 Bee carrying pollen in pollen basket on its legs(coutesy Min House Epiery Kota Bharu Kelantan)



Fig 6: Commercially produced beebread for human consumption(product of Apis Vita)

1.2 Problem Statement

Malnutrition remains a major source of post-operative complications including wound break down and wound infections due to poor wound healing [Sungurtekin H, Sungurtekin U, Balci C et al 2004]. Enteral nutritional supplementation has been shown to promote wound healing.[Wallace E. Br J Nurs 1994].In this era of natural bio products Bee bread, being a natural bio product containing all the important essential food elements including carbohydrate,protein,essential fatty acids,vitamins,minerals and trace elements; will be an ideal enteral nutritional supplement in malnourished surgical patients in order to promote good wound healing.

Poor wound healing resulting in wound break down and wound infection constitute a major source of post-operative mortality and morbidity as well as increased duration of hospital stay with its attendant increased financial burden on both patients and the healthcare system. Recent studies have shown a rising trend in the enormous financial burden of wound management on the community running in millions of dollars (about \$286m per anum) in cost of hospital bed days only in Australia - Arana V. Biomed Pharmacotherapy(2004); PUPPS 3(2006). Statewide report.

Wound could be a source of morbidity and mortality due to inflammation and infection with subsequent release of cytokines and other inflammatory mediators resulting in systemic infection, multi organ failure (MOD) and death.

Nutrition plays an important role in wound healing thus the need for nutritional supplementation in surgical patient who are malnourished. Malnourished patients have been found to have higher rate of wound related complications such as wound break down including burst abdomen and wound infection with associated increased morbidity and mortality of up to 20 % (Demling RH. Adv Skin Wound Care (2006). Pre and post-operative nutritional supplementation has been shown in several studies to promote good wound healing with significant reduction in wound related post-operative complications (Williams JZ (2003), Surg Clin ,N Am Wallace E(1994),. Br J Nurs

Literature Review

2.1. Wound healing:

Wound healing is a tissue response to injury either intentional (e.g. surgical) or accidental (e.g. burns), which involves increased cellular activity, synthesis of new proteins and increased tissue energy consumption.

Complete wound healing consists of four overlapping phases: haemostasis, inflammatory, proliferative and remodeling phase. This takes about 2 to 3 weeks to complete. Some wounds fail to heal in a timely and orderly manner, resulting in chronic non-healing wounds. Alterations in one or more of these components could account for the impaired healing observed in chronic wounds because cytokines, growth factors, proteases, and cellular and extracellular elements all play important roles in different stages of the healing process which is in turn affected by several factors such as nutritional status, infections malignancies diabetes etc. Also, dysregulation in certain stages of the healing process could result in excessive deposition of collagen and formation of abnormal scar, as seen in hypertrophic scars and keloids (Stuart Enoch 2008).

2.2. Role of nutrition and nutritional supplementation in wound healing

Many studies have established the significance of nutritional status of patient in healing (Cathy Alberda, Linda McCargar et al 2008). All food components such as protein carbohydrates fatty acids, vitamins especially vit A and vit C, minerals and trace elements

have been shown to play vital role in various stages of wound healing(Cohen IK et al 1992;Trumbo et al(2001).

For proper wound healing to take place in malnourished patients, nutritional supplementation must be instituted appropriately. This can be administered enterally or parenterally.Pareteral nutrition is associated with many complications such as phlebitis, thromboembolism, cellulitis and sepsis. As a result the gold standard is to administer nutritional supplementation enterally except where contraindicated (Jeejeebhoy KN.et al 2005; Gramlich L, Kichian et al 2004)

2.3. Beebread as Nutritional Supplement

Beebread being a naturally occurring bio product with high nutritional content has been recommended for human consumption as a source of high energy and protein (Kroyer and Hegedus 2001; Campos et al 2003).

There is a growing demand of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and to the increased consumer perception of this problem in recent years(Javanmardi et al 2002; Vilma Baltrusaityte et al 2006).Thus beebread will be a suitable candidate in the future of pre and post-operative nutritional supplementation for optimization of wound healing

2.4 Bactericidal effect of beebread

In a study by Z.Abouda et al (2010),the antibacterial effect of beebread was demonstrated.The study showed that solutions of beebread extract has bactericidal effect on both gram positive and gram negative bacteria.Gram positive bacteria showed more sensitivity in the experiment.

2.5 The anti-oxidant activity of beebread

The anti-oxidant activity of beebread is mainly due its scavenging activity on oxygen radicals. This action of beebread was demonstrated in a study by Nagai *et al.*, (2004).

This property of beebread has been used with some effectiveness in the treatment of cancer, hypertension and diabetes

2.6 Beebread as an immune booster

Ishikawa *et al* (2008), in his study has demonstrated the medicinal properties of beebread including its immunostimulatory effects. Bee pollen is widely used for ailments such as chronic prostatitis for its anti-inflammatory activity (Wagenlehner *et al.*, 2009). Shoskes *et al* (2002) has demonstrated the anti-androgenic effects of beebread. Other studies have shown the anti-tumor activity (Yang *et al.*, 2007), as well as its anti-allergic effect (Medeiros *et al.*, 2008).

2.6 Beebread as a multivitamin and mineral supplement

Beebread contains a wide range of multivitamins as well as mineral. A study carried out by G. Stanciu *et al* (2009) in which he investigated the chemical content of beebread from different regions, concluded that beebread contains a complete range of vitamin and mineral essential for body metabolisms.

2.4. Assessment of Wound Healing

The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient giving rise to a fine scar with high tensile strength.

Effectiveness of wound healing can be assessed by measuring tensile strength, fibroblasts assessment, and collagen or hydroxyproline content of the wound among other factors.

One of the most important factors in the healing of wounds is the stimulation of wound strength. Wound strength is determined by the amount and quality of newly synthesized and deposited collagen, as well as degradation of preformed collagen. Tensile strength, which is demonstrated by the force per unit of cross-sectional area needed to break the wound, is an important measure since it reflects the sub dermal organization of the collagen fibers in the newly deposited collagen as shown in a study by M.Z. Rozaini, A.B.Z. Zuki et al 2004. Tensile strength indicates how much the repaired tissue resists to break under tension and may indicate in part the quality of the repaired tissue. Collagen is one of the major components that is mainly responsible for the mechanical properties of the skin

Fibroblasts are the main cellular component responsible for extracellular matrix deposition and collagen synthesis and thus their assessment can determine the quality of wound healing as indicted in study by Nisbet et al in 2010. In this study rabbits treated with honey show significant improvement in healing properties which includes assessment of fibroblasts and hydroxyproline content of the wound tissue. Proline is an important constituent of tissue protein including collagen. Its hydroxylation increases the

stability and strength of collagen and by extension that of healing wound (Nisbet et al 2010).

3.0. Research Objectives

3.1. General Objective3s:

To examine the effect of beebread supplementation on wound healing in malnourished rabbits.

3.2 Specific Objectives:

1. To determine the tensile strength postt operative wound of malnourished rabbts supple
- mented with beebread
- 2 To determine the fibroblasts content of the post-operative wound of malnourished
Rabbits supplemented with beebread
- 3 To determine the epithelization of the post-operative wound of malnourished rabbits
supplemented with beebread
- 4 To determine the neovascularization of the post-operative wound of malnourished
rabbits supplemented with bee bread
- 5 To determine the level of hydroxyproline in surgical wound of malnourished rabbits
after supplementation with beebread

3.3. Research Hypothesis:

The median outcome of wound healing and tensile strength of malnourished rabbits given oral beebread is better compared to the control group given standard rabbit pellet.

3.4. Justification of the Study:

Beebread is a naturally occurring product that has been adapted as a nutritional supplement. There is growing demand for natural products in human diet, thus investigating its application in nutritional supplementation of surgical patient at this time is appropriate.

METHODOLOGY

4.1 Study Design

This is a pilot study on the effect of beebread on wound healing conducted as a randomized controlled animal study.

Randomisation of Animals

- At the age of 6 to 10 weeks old, 30 New Zealand white rabbits, weighing about 2.5 to 3kg (+/- 300g) were classified and scheduled for the study.

The rabbits were kept in animal research and service center (ARASC) USM, and feed with free access to standard commercial diet and water throughout the study. The rabbits were kept individually per cage.



Fig 7: Rabbits in individual cages

The animals were then distributed using block randomization into two groups of 12 each and then submitted to the experimental protocols which was conducted in two phases; phase 1 : Induction of malnourishment and phase II experimental laparotomy

Phase I ; Induction of malnourishment

The rabbits were induced to become malnourished as described below:

- After 3 days being fed with normal rabbit die *tad libitum*, their baseline body weights were determine on a scale with precision of 1 gram, then the animal were subjected to malnutrition induction :

A preliminary study was conducted to determine the appropriate amountof diet reduction that will produce the desired gradual weight reduction and finally give a steady 10% reduction in body weight. Diet reduction of 10%,20% ,40%,50% and 60%were used. The

10%,20%and40% reduction in diet did not produce significant weight reduction. The 60% produced excessive weight reduction that did not stabilizeThe 30% reduction in diet gave the desired result and was adapted.

To establish a malnourished state the 30 rabbits were fed daily for 30 days with half the amount of food that a well-nourished group had consumed on the previous days.

Daily Average weight of 24 rabbits

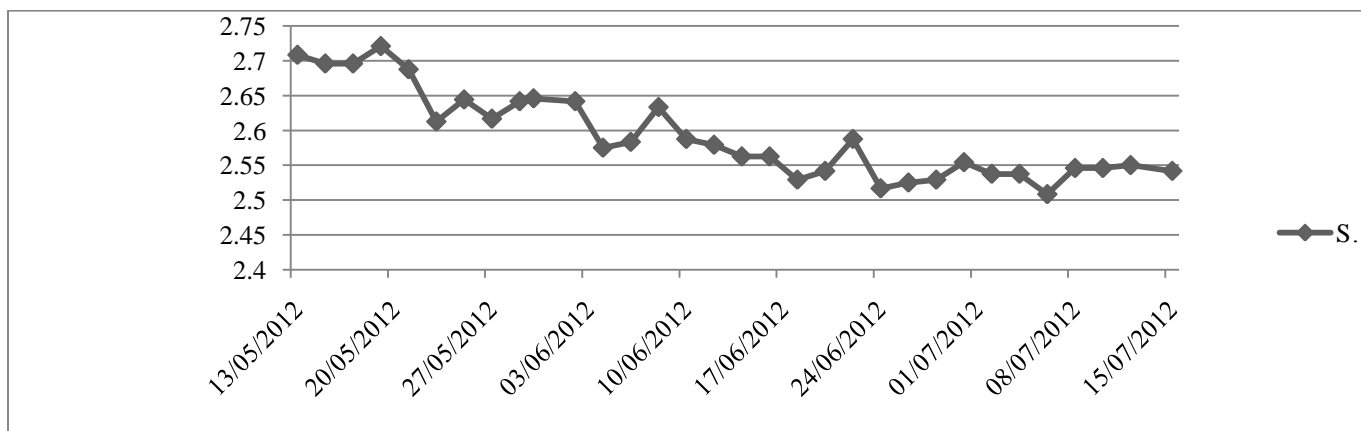


Chart 1; Weight Reduction Graph

- The body weight of each rabbits was monitored every morning. Malnutrition state was achieved when the rabbits have lost 10% body weight and reached a static body weight

- Base line data on 6 rabbits normal nourished control group was collected in which the 6 rabbits were fed normal diet and subject to laparotomy.

Phase II: Experimental laparotomy

After 30 days of scheduled malnourishment, the 24 malnourished rabbits were subjected to laparotomy

Surgical technique:

12 hours before surgery the food is withdrawn, but water is given ad libitum.

- The rabbits were premedicated with IM xylazine (7mg/kg) and anaesthetized using intramuscular injection of ketamine 40mg/ kg body weight. The rabbit are placed in prone position and immobilize on their back for the surgery. Inhalational anaesthesia was used for maintenance using Isoflourane and oxygen.
- When fully anaesthetized, the shaved areas were cleaned with povidone iodine, alcohol 70 % and Hibiscrub®. The operation site is isolated with sterile towel. Amidline laparotomy was performed using a surgical blade size 15.
- The abdominal musculo-aponeurotic layer was closed with 4-0 polypropylene continuous sutures. The skin and subcutaneous tissue layer was closed with 4-0 dafilon interrupted suture.



Fig 8 :Anaesthesia being administered.



fig 9:Anterior abdominal wall being cleaned

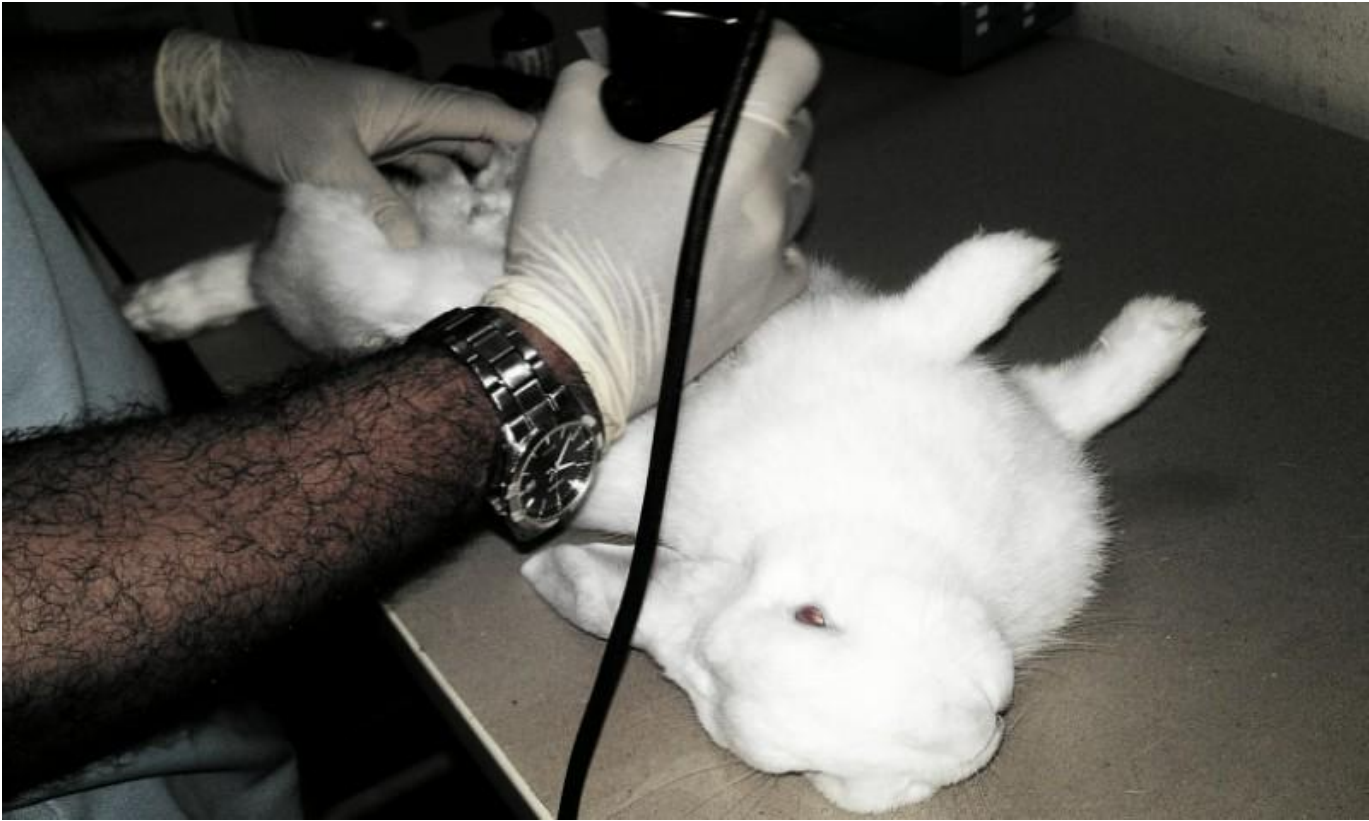


Fig 10: Anterior abdominal wall is being shaved



Fig 11: Operation site is cleaned with povidone iodine and alcohol



Fig 12 Anesthetic Machine used for the procedure



Fig: 13 Anaesthesia being administered to one of the rabbits



Fig: 14 The rabbit is placed in supine position for the laparotomy

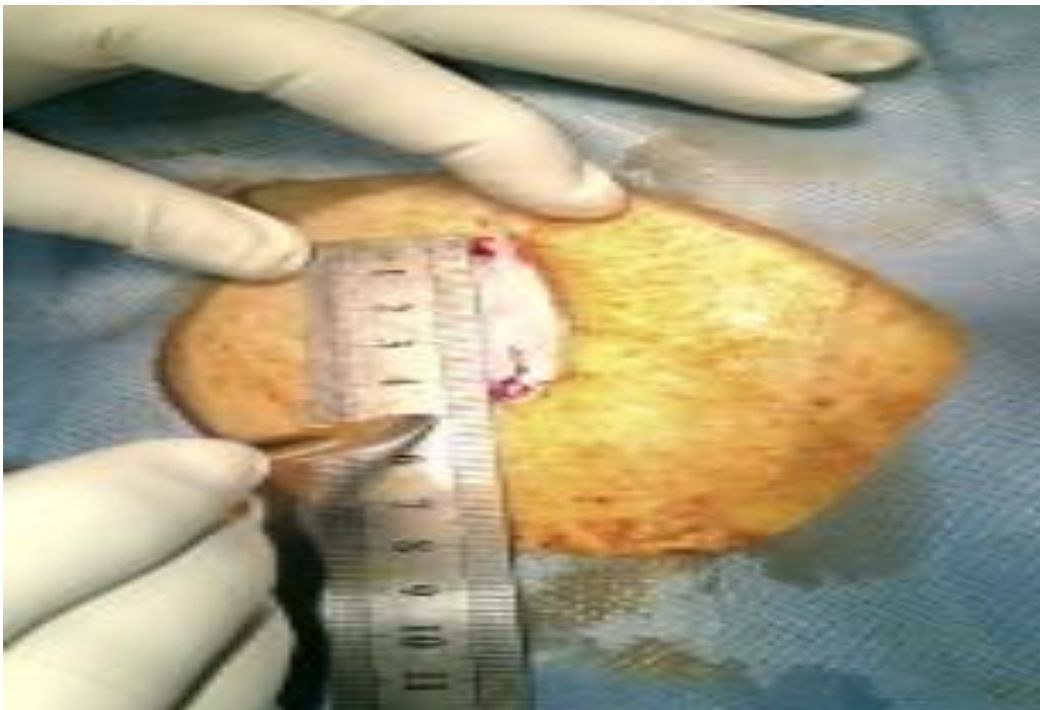


Fig15: A 4cm midline incision was made under aseptic technique



Fig 16: The musculofacial layer of the anterior abdominal wall is being closed with 4/0 propylene suture

The animals were monitored immediately post-operatively for spontaneous breathing effort and movement. Post operatively, analgesic will be given: i.m.Buprenorphine 0.01-0.05 mg/kg 12 hourly at least for 3 days.

- During 8 hours after surgery the rabbits were offered water. After this period the diet was changed to their normal diet for 12 rabbits which are the control group;and 70% normal diet with 30% beebread for 12 rabbits which are the treatment group, ie 24 rabbits of malnourished state.

Randomizations:

Table of Randomization

Group 1 (12 malnourished rabbits)	Control group., received standard rabbit diet(post lapaoatomy).6 rabbits were saccrificed on day 7 post surgery,another six saccrificed dady 14 post surgery for tissue harvesting.
Group 2 (12 malnourished rabbits rabbits) Study group	Supplemented group, receive 70% normal rabbit diet + 30% of diet as beebread spplementation(post laparotomy). 6 rabbits were saccrificed day 7 post surgery,another 6 were saccrifised day 14 post surgery f0r tissue harvesting.

The researcher is blind towards the group of rabbits.