THE EFFECT OF KELULUT HONEY ON CLINICAL, INFLAMMATORY AND IMMUNOLOGICAL RESPONSE IN PATIENTS UNDERGOING LOWER GASTROINTESTINAL SURGERY: A PILOT STUDY

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Finally, I would like to thank my parents, siblings, Bailey and close friends. With their endless support and advice, this task has gotten easier to complete. Thank you very much.

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LIST OF ABBREVIATIONS

1. CBS: Capillary Blood Sugar
2. CRP: C-Reactive Protein
3. IL-1: Interleukin-1
4. IL-6: Interleukin-6
5. SSI: Surgical Site Infection
6. TNF-α: Tumour Necrosis Factor α
7. NRS Score: Nutrition Risk Screening Score
8. HILDA: Honey Interlinked Dehydration and Dispenser Apparatus
9. MUSTAFA-Hive: Meliponiculture Using Split-able Throne within Air-jacketed Palace for Amplification-Hive.

ABSTRACT

Background: *Kelulut* honey is a type of honey produced by stingless bee. It exhibits anti-inflammatory and anti-oxidant properties and consists of fructose, glucose and trehalulose. These properties could potentially lessen the inflammatory response after surgery. To date, there is no clinical study conducted specifically to assess the effect of *Kelulut* honey in patients undergoing lower gastrointestinal surgery.

Method and Material: We conducted a pilot study involving 42 participants undergoing lower gastrointestinal surgery. Participants were randomised into *Kelulut* honey and Carborie® (control) group. Participants were required to consume 500-ml study beverage equivalent to 235 Kcal, 3 times daily on post-operative days 1 and 2. Primary endpoints were the time of first flatus and first bowel evacuation and CRP level on admission, 12^{th}, 36^{th}, 60^{th} and 96^{th} hour post-operatively. CBS was monitored in diabetic patients. Secondary endpoints were incidence of nosocomial infection, SSI and length of stay.

Results: 2 participants withdrew before the surgery, leaving only 40 participants with equal size group. First flatus was reported after 28.5 hours and 37.0 hours post-operatively in *Kelulut* honey and Carborie® group respectively. This difference was not statistically significant (P= 0.493). First bowel evacuation occurred earlier in *Kelulut* honey group at 47.92 hours, while that of control was at 65.78 hours post-operatively. This difference is not statistically significant (P=0.180). Serial CRP level measurement on 12^{th}, 36^{th}, 60^{th} and 96^{th} hour post-operatively was lower in the *Kelulut* honey group as compared to that of control, however it was not statistically significant. (P= 0.246, 0.884, 0.937, and 0.730). There was no difference demonstrated in incidence of nosocomial infection, SSI and length of stay. 15% of diabetic participants (n=11) in *Kelulut* honey group had persistent hyperglycaemia until day 4.

Conclusion: Oral consumption of *Kelulut* honey after lower gastrointestinal surgery is safe. While there was no significant difference observed in between 2 groups, further study with larger sample size would be required to associate *Kelulut* honey with lower CRP levels.

ABSTRAK

Latar belakang: Madu Kelulut adalah sejenis madu yang dihasilkan oleh lebah Kelulut. Ia mempunyai ciri anti-radang dan antioksidan serta terdiri daripada fruktosa, glukosa dan trehalulosa. Ciri-ciri ini menjadikan ia berpotensi untuk mengurangkan tindakbalas keradangan selepas pembedahan. Pada hari ini, belum ada kajian klinikal yang secara spesifik menilai kesan madu Kelulut terhadap pesakit yang menjalani pembedahan usus.

Kaedah Kajian dan Bahan: Kami menjalankan kajian rintis melibatkan 42 orang pesakit yang menjalani pembedahan usus. Para peserta telah secara rawak dibahagikan kepada kumpulan madu Kelulut dan Carborie® (kawalan). Para peserta dikehendaki meminum sajian minuman khusus kajian ini yang berjumlah 500 ml bersamaan 235 Kcal, 3 kali sehari pada hari pertama dan kedua selepas pembedahan. Titik akhir utama kajian ini adalah masa kentut dan masa penyahtinjaan yang pertama serta paras CRP semasa kemasukan, 12, 36, 60 dan 96 jam selepas pembedahan. Paras gula (CBS) akan dipantau bagi penghidap kencing manis. Titik akhir kedua adalah kejadian jangkitan nosokomial, jangkitan luka pembedahan serta tempoh penginapan.

Keputusan: 2 peserta telah menarik diri sebelum pembedahan, menjadikan hanya 40 peserta yang tinggal dalam saiz kumpulan yang sama. Kentut yang pertama dilaporkan selepas 28.5 jam dan 37.0 jam selepas pembedahan masing-masing untuk kumpulan madu Kelulut dan kumpulan Carborie®. Perbezaan ini secara statistiknya tidak ketara (P=0.493). Masa penyahtinjaan yang pertama berlaku lebih awal di kumpulan madu Kelulut pada 47.92 jam , sementara masa tersebut di kumpulan kawalan adalah pada 65.78 jam selepas pembedahan. Perbezaan ini juga tidak ketara (P=0.180). Catatan bersiri paras CRP pada 12, 36, 60 dan 96 jam selepas pembedahan adalah lebih rendah di kumpulan madu Kelulut jika dibandingkan dengan kawalan, walaubagaimanapun perbezaan ini secara statistiknya tidak ketara (P=0.246, 0.884, 0.937, and 0.730). Tiada perbezaan ditunjukkan dalam kejadian jangkitan nosokomial, jangkitan luka pembedahan dan tempoh penginapan. 15% daripada penghidap diabetes (n=11) di kumpulan madu Kelulut mengalami paras gula tidak terkawal yang berterusan hingga hari ke-4.

Kesimpulan: Meminum madu Kelulut selepas pembedahan usus adalah selamat. Meskipun tiada perbezaan ketara dapat diperhatikan di antara kedua-dua kumpulan ini, kajian lanjut diperlukan dengan saiz sample yang lebih besar untuk mengaitkan madu Kelulut dengan paras CRP yang rendah.

CHAPTER 1: INTRODUCTION

1.1. INTRODUCTION & LITERATURE REVIEW

Kelulut Honey is a type of honey produced by Meliponine sp or known as stingless bee. This type of honey is found in tropical and sub-tropical regions, including Malaysia. It is different from other kind of honey, known by its distinctive sweet-sour taste with minimal crystallisation. Just like any other kind of honey, it consists mainly of fructose and glucose (1-3). However, *Kelulut* honey is unique in the sense that it contains a higher proportion of trehalulose which an isomer of sucrose with an unusual glucose-fructose linkange. This is the reason behind its lower glycaemic index and acariogenic properties. Its trehalulose content ranges between 13 – 44 g in each 100 g of *Kelulut* honey (4). In fact, all honey in general, cause lesser elevation of plasma glucose level, insulin, C-Peptide and CRP when compared with dextrose (5). In a small-sized study, it was also reported that daily intake of 30 g *Kelulut* Honey for a month resulted in insignificant effect on fasting blood glucose, fasting lipid profiles, and other metabolic parameters (6). This low glycaemic property has made *Kelulut* honey suitable and safe to be consumed by diabetic individuals. Among most notable character of *Kelulut* honey is its anti-inflammatory and antioxidant properties. When compared to other types of honey, *Kelulut* Honey is found to have higher proportion of polyphenols. The polyphenols found are mainly flavonoid, phenolic acids and phenolic acid derivatives. This property has proven to be beneficial for wound healing (7,8). *Kelulut* Honey also contains small amounts of proteins, amino acids, enzymes, vitamin, and mineral. Anti-bacterial properties of honey including *Kelulut* Honey was also reported (3). Among a concern often raised in the community is the quality and contamination of the honey. This can be explained by our tropical climate with high humidity level and thus resulting in honey with high moisture content, which

will lead to fermentation. With invention of an apparatus called HILDA (Honey Interlinked Dehydration and Dispenser Apparatus) which allows *Kelulut* Honey to undergo dehydration without heating, this problem has significantly declined. The apparatus allowed dehydration of honey from 35% to around 18%, and therefore prevent fermentation and contamination (2). Production of *Kelulut* honey that is hygienic and medically safe was also made possible with the MUSTAFA-Hive (Meliponiculture Using Split-able Throne within Air-jacketed Palace For Amplification-Hive). Our *Kelulut* honey industry is undergoing continuous expansion with extensive academic research as well as monitoring of its standard to ensure its quality. The *Kelulut* standard MS2683:2017 is a cornerstone in ensuring the quality and safety of the *Kelulut* Honey. (10) At present, the spectrum of medical use of honey remains limited to topical application. While it is mostly consumed orally as functional foods, its consumption during peri-operative periods is almost unheard of. Its nutritional value alongside its anti-inflammatory effects is something that is worth to pay attention into as these could potentially reduce the magnitude of stress response following surgery.

It is good to recall that, surgery, while it is intended to treat an illness, is itself a form of stress to the body. As surgery begins, cascades of immunological response mediated by various hormonal and metabolic pathways area activated, and thus systemic inflammatory responses occur. This mechanism is there to protect the body and at the same time prepare it for initiation of healing process. The magnitude of this inflammatory response following surgery can sometimes be overwhelming, that it can do more harm than good to the body. It is this response that we are aiming at to modulate so that the recovery process is smooth and timely. The main idea behind the early recovery program was to modulate this inflammatory response by several multimodal strategies which

include optimal analgesia, neural blockade, minimally invasive surgery, pre-operative carbohydrate loading as well as early oral nutrition. (11)

Behind the scenes, glucagon and catecholamines elevation occurs during surgical stress and this eventually exhausts the hepatic glycogen reserve and triggering gluconeogenesis, which may be accompanied by insulin resistance. Release of pro-inflammatory cytokines such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), and tumour necrosis α (TNFα) occur soon after the incision. IL-6 is responsible for the synthesis of acute-phase proteins such as C-Reactive Protein (CRP) (12,13). These markers of inflammatory states have different kinetics. IL-6 level increases and peaks several hours after surgery. The magnitude is highly influenced by type of surgery and the degree of stress it evokes. Higher level is observed in pelvic and colorectal surgery as well as vascular surgery (14). In one study, IL-6 was observed to reach its peak 2 hours after the surgery (15). IL-6 should have reached its peak within 24 hours after surgery and therefore it should return towards its baseline on post-operative day 2 (16,17). It was concluded in one study that IL-6 level of more than 432 pg/ml on post-operative day 1 is associated with higher risk of complications (18). CRP elevation occurs in response to abundance of pro-inflammatory cytokines. Unlike IL-6, it peaks at around 48 hours after surgery (18-21). It was reported that median level of post-operative CRP in the absence of inflammatory complications were 94.5 mg/L on Day 1, 142.0 mg/L on Day 2, 123.0 mg/L on Day 3, 78.0 mg/L on Day 4, and 56.5 mg/L on Day 5. CRP level of 123 mg/L on post-operative day 4 is a cut-off value for CRP to discriminate those with or without inflammatory complications (20). If CRP is to be interpreted on day 3 to predict inflammatory complication, it was suggested that CRP level of 203 mg/L would be discriminative enough for increased risk of complications (18).

To put things into perspective, we are investigating the effect of *Kelulut* honey in patients undergoing lower gastrointestinal surgery. The anti-inflammatory effects are to be examined as well as whether it makes difference in modulating post-operative ileus. As there are abundance of evidence to support early oral nutrition, we would adopt the same strategy by using *Kelulut* honey (11,23-29). In fact, delay in resumption of feeding post operatively is associated with more infectious complications and delayed recovery (30-32).

1.2. STUDY JUSTIFICATION

The oral consumption of *Kelulut* honey or any other types of honey during post-operative periods are not commonly practiced. It may be consumed by patients who are familiar with the honey and this, most of the time, is a matter of patient's personal preference rather than a recommendation from the expert. Even though honey is safe to be consumed, some patients and clinical practitioners are still doubtful whether its oral consumption during post-operative periods will interfere with recovery process. To date, there is no clinical study conducted specifically to assess the effect of *Kelulut* honey.

While early oral nutrition is important in promoting post-operative recovery, the choice of nutrition to consume in early post-operative periods may not be abundant. It is generally accepted that oral diet would begin with introduction of liquid diet in the form of clear fluid, but the definition may be different from one clinician to another. Clear fluid is not equal to just a plain water, but in definition, it is a solution which contains easily digested molecules, leaving no undigested residue in gastrointestinal tract. In our post-operative practice, we tend to begin with clear fluid. While there is a need to provide at least around 50% of total required calorie requirement per day so that the stress response may be attenuated, but in principle, this must be balanced with the patient's appetite and

desire, post-operative ileus and post-operative nausea and vomiting. In fact, one of major concern in the past that early oral nutrition may aggravate the ileus but there is more evidence now that refuted this idea (26,27,30). Once the clinical progress shows that it is safe to step up the feeding, more complex diet can be introduced, and eventually a normal diet can be resumed days later. Correlating with this common scenario, *Kelulut* honey is one of a good choice of clear fluid. It is composed mainly of fructose, glucose and trehalulose, which are rapidly absorbed and leaving no residue in the gastrointestinal tract. For now, one of the common clear fluids in the market is Carborie® by Valens. It is commercially manufactured for the use during peri-operative periods and is composed of Maltodextrin derived from corn. However, it is costly, and is not cost-effective in the long run. Other types of clear fluid in the market may be cheaper and can be found in the hospital mart, but they tend to undergo multiple processing and are either containing too little energy or contain high glycaemic index carbohydrate. *Kelulut* honey on the other hand is a natural source of simple sugars, that possesses low glycaemic index. This is a positive point that makes it safe in diabetics. In addition, its higher concentration of polyphenols that contribute to its anti-inflammatory properties is potentially helpful in improving the degree of inflammatory response after surgery, and thus improving the outcome. Before a recommendation can be made on its use in clinical practice, we conducted a clinical study on the effects of *Kelulut* honey on clinical, inflammatory and immunological response in patients undergoing lower gastrointestinal surgeries. The clinical endpoints would be that of bowel motility reflected by time of first flatus and first bowel evacuation and immunological endpoints are the level of post-operative CRP obtained by serial measurement.

1.3. RESEARCH QUESTION(S)

1. Does the use of *Kelulut* Honey given post-operatively facilitate earlier return of gastrointestinal function?
2. Does *Kelulut* Honey significantly improve post-operative inflammatory and immunological markers and therefore reduce risk of post-operative complications?

1.4. OBJECTIVES

General Objectives:

To determine the effect of *Kelulut* Honey consumed in the early post-operative period on the return of bowel functions and on the post-operative inflammatory and immunological markers in patients undergoing lower gastrointestinal surgery.

Specific Objectives:

1. To compare the time of the first flatus and first bowel evacuation between *Kelulut* Honey and Carborie® when consumed in the early post-operative period after lower gastrointestinal surgery.
2. To associate the change of C-Reactive Protein (CRP) pre- and post-operatively in patients consuming *Kelulut* Honey versus Carborie® in the early post-operative period after lower gastrointestinal surgery.

1.5. RESEARCH HYPOTHESIS

First Hypothesis

H_0: There is no significant difference between the time of the first flatus and first bowel evacuation between *Kelulut* Honey and Carborie® when consumed in the early post-operative period after lower gastrointestinal surgery.

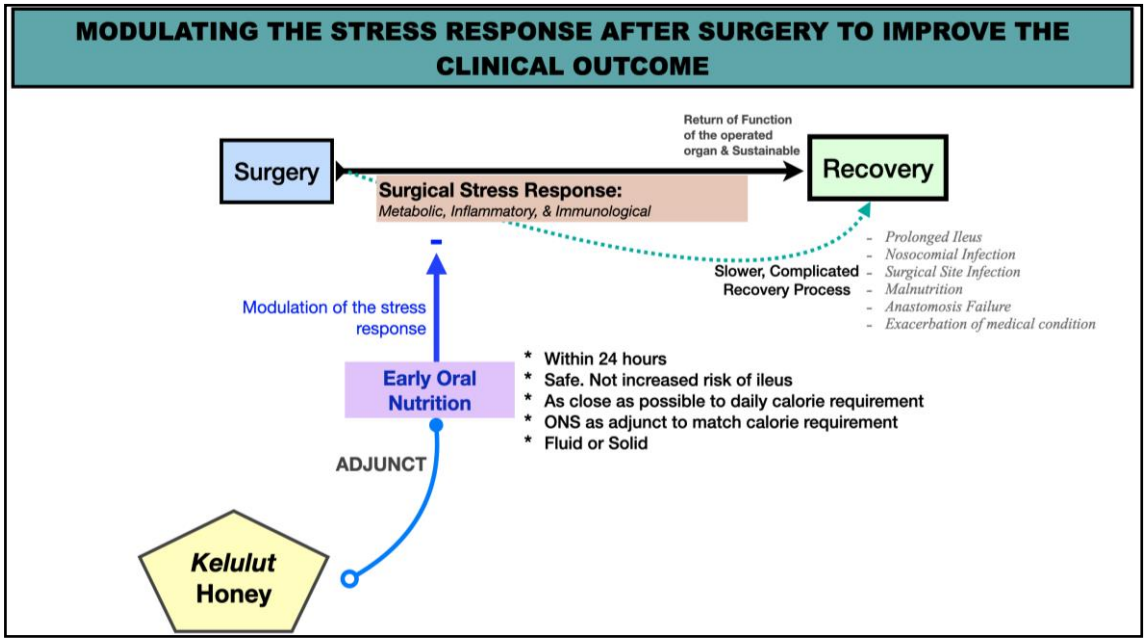
H_1: There is a significant difference between the time of the first flatus and first bowel evacuation between *Kelulut* Honey and Carborie® when consumed in the early post-operative period after lower gastrointestinal surgery.

Second Hypothesis

H_0: There is no significant difference between the level of C-Reactive Protein (CRP) pre- and post-operatively in patients consuming *Kelulut* Honey versus Carborie® in the early post-operative period after lower gastrointestinal surgery.

H_1: There is a significant difference between C-Reactive Protein (CRP) pre- and post-operatively in patients consuming *Kelulut* Honey versus Carborie® in the early post-operative period after lower gastrointestinal surgery.

1.6. CONCEPTUAL FRAMEWORK



1.7. APPROVAL FROM HUMAN RESEARCH ETHICS COMMITTEE (HREC) USM

Jawatankuasa Etika
Penyelidikan Manusia USM (JEPeM)

Human Research Ethics Committee USM (HREC)

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14th December 2021

Dr. Mohammad Izwan Mohd Isa
Department of Surgery
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JEPeM Code : USM/JEPeM/21080536
Protocol Title: The Effect of Kelulut Honey on Clinical, Inflammatory and Immunological Response in Patients Undergoing Lower Gastrointestinal Surgery: A Randomised Controlled Study.

Dear Dr.,

We wish to inform you that your study protocol has been reviewed and is hereby granted approval for implementation by the Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia (JEPeM-USM). Your study has been assigned study protocol code **USM/JEPeM/21080536**, which should be used for all communications to JEPeM-USM in relation to this study. This ethical approval is valid from **14th December 2021** until **13th December 2022.**

Study Site: Hospital Universiti Sains Malaysia.

The following researchers are also involved in this study:
1. Assoc. Prof. Dr. Andee Dzulkarnaen Zakaria
2. Dr. Mohd Azem Fathi Mohammad Azmi
3. Dr. Mohd Zulkifli Mustafa
4. Dr. Nurul Khaiza Yahya
5. Assoc. Prof. Dr. Najib Majdi Yaacob

The following documents have been approved for use in the study.
1. Research Proposal

In addition to the abovementioned documents, the following technical documents were included in the review on which this approval was based:
1. Patient Information Sheet and Consent Form (English version)
2. Patient Information Sheet and Consent Form (Malay version)
3. Data Collection Form

The list of JEPeM-USM members present during the full board meeting reviewing your protocol is attached.

While the study is in progress, we request you to submit to us the following documents:
1. Application for renewal of ethical approval 60 days before the expiration date of this approval through submission of **JEPeM-USM FORM 3(B) 2019: Continuing Review Application Form.**
2. Any changes in the protocol, especially those that may adversely affect the safety of the participants during the conduct of the trial including changes in personnel, must be submitted or reported using **JEPeM-USM FORM 3(A) 2019: Study Protocol Amendment Submission Form.**
3. Revisions in the informed consent form using the **JEPeM-USM FORM 3(A) 2019: Study Protocol Amendment Submission Form.**

4. Reports of adverse events including from other study sites (national, international) using the **JEPeM-USM FORM 3(G) 2019: Adverse Events Report.**
5. Notice of early termination of the study and reasons for such using **JEPeM-USM FORM 3(E) 2019.**
6. Any event which may have ethical significance.
7. Any information which is needed by the JEPeM-USM to do ongoing review.
8. Notice of time of completion of the study using **JEPeM-USM FORM 3(C) 2019: Final Report Form.**

Please note that forms may be downloaded from the JEPeM-USM website: **www.jepem.kk.usm.my**

JEPeM-USM is in compliance with the Declaration of Helsinki, International Conference on Harmonization (ICH) Guidelines, Good Clinical Practice (GCP) Standards, Council for International Organizations of Medical Sciences (CIOMS) Guidelines, World Health Organization (WHO) Standards and Operational Guidance for Ethics Review of Health-Related Research and Surveying and Evaluating Ethical Review Practices, EC/IRB Standard Operating Procedures (SOPs), and Local Regulations and Standards in Ethical Review.

Thank you.

"WAWASAN KEMAKMURAN BERSAMA 2030"

"BERKHIDMAT UNTUK NEGARA"

Sincerely,

DR. NOOR AMAN A. HAMID
Deputy Chairperson
Jawatankuasa Etika Penyelidikan (Manusia), JEPeM
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Date of meeting : 23rd September 2021
Venue : Through WEBEX Application
Time : 9.30 a.m – 2.00 p.m
Meeting No : 517

Members of Committee of the Jawatankuasa Etika Penyelidikan (Manusia), JEPeM Universiti Sains Malaysia who reviewed the protocol/documents are as follows:

	Member (Title and Name)	Occupation (Designation)	Male/ Female (M/F)	Tick (✓) present during review process
Deputy Chairperson: Dr. Noor Aman A. Hamid		Deputy Chairperson of Jawatankuasa Etika Penyelidikan (Manusia), JEPeM USM	M	✓ **(Deputy Chairperson)**
Secretary: Mr. Mohd Bazlan Hafidz Mukrim		Science Officer	M	✓
Members :				
1.	Assoc. Prof. Dr. Adibah Ibrahim	Lecturer, School of Medical Sciences	F	✓
2.	Assoc. Prof. Dr. Garry Kuan Pei Ern	Lecturer, School of Health Sciences	M	✓
3.	Assoc. Prof. Dr. Haslina Taib	Lecturer, School of Dental Sciences	F	✓
4.	Dr. Juhaida Daud	Representative of Hospital USM	F	✓
5.	Dr. Michael Wong Pak Kai	Lecturer, School of Medical Sciences	M	✓
6.	Prof. Dr. Nik Hazlina Nik Hussain	Lecturer, School of Medical Sciences	F	✓
7.	Mrs. Norleha Mohd Noor	Executive Secretary, Office of Director of Hospital USM	F	✓
8.	Assoc. Prof. Dr. Norsarwany Mohamad	Lecturer, School of Medical Sciences	F	✓
9.	Prof. Dr. Suzina Sheikh Ab Hamid	Lecturer, School of Medical Sciences	F	✓
10.	Mrs. Zawiah Abu Bakar	Community Representatives	F	✓

Jawatankuasa Etika Penyelidikan (Manusia), JEPeM-USM is in compliance with the Declaration of Helsinki, International Conference on Harmonization (ICH) Guidelines, Good Clinical Practice (GCP) Standards, Council for International Organizations of Medical Sciences (CIOMS) Guidelines, World Health Organization (WHO) Standards and Operational Guidance for Ethics Review of Health-Related Research and Surveying and Evaluating Ethical Review Practices, EC/IRB Standard Operating Procedures (SOPs), and Local Regulations and Standards in Ethical Review.

DR. NOOR AMAN A. HAMID
Deputy Chairperson
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CHAPTER 2: STUDY PROTOCOL

2.1. INTRODUCTION AND LITERATURE REVIEW

Surgery is a form of stress to the body which triggers series of clinical and biochemical responses, which are of protective value to the body and at the same time serve as platform for initiation of healing processes. It is the activation of hypothalamo-pituitary-adrenal axis in addition to stimulation of sympathetic nervous system that led to hormonal, metabolic, inflammatory, and immunological response. It is the magnitude of these responses which determine outcome of the surgery. The stress response is parallel to the degree of the tissue injury. Of note, these stress responses can be overwhelming in susceptible individuals and lead to undesirable outcome. Clinical management during peri-operative periods are directed towards modifying the magnitude of these response so that the post operative complications, morbidity and mortality rate can be minimised. It is therefore mandatory to have an adequate pre-operative preparation and optimal post-operative management in ensuring a satisfactory outcome.

The stress response following a surgery triggers elevation of glucagon and catecholamines in the plasma, which in turn cause exhaustion of the hepatic glycogen reserve. Gluconeogenesis ensues and hepatic glucose production occurs by using non-glucose substrates such as gluconeogenic amino acids, glycerol, and lactate. All these are directed towards increasing glucose concentration in the plasma to fuel the increased energy demand, but at the expense of protein catabolism. However, hormonal, and inflammatory mediators trigger insulin resistance, which lead to reduced glucose oxidation and hyperglycaemia. For this reason, the body shifts into fatty acids to meet the increasing energy demand and therefore fat becomes major source of fuel during post

operative period. At the same time, skeletal muscle protein breakdown allows reprioritisation of protein synthesis to acute phase reactants such as C-Reactive Protein (CRP) and Fibrinogen, which are the hallmark of the inflammatory state. The innate and adaptive immune system work in synergism in co-generating the metabolic and inflammatory response. This is evident by significant elevation of pro-inflammatory cytokines such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), and tumour necrosis α (TNFα) after surgery. IL-6 is responsible for the hepatic component of inflammatory response following surgery, resulting in the synthesis of acute-phase proteins.[1,2.] Understanding the kinetics of these markers, especially those of IL-6 and CRP, is of the utmost importance in the anticipation of post-operative complications.

IL-6 level increases and peaks several hours after surgery. The magnitude is highly influenced by type of surgery and the degree of stress it evokes. Higher level is observed in pelvic and colorectal surgery as well as vascular surgery.[3] In resection of colorectal cancer, it is observed that IL-6 peaked 2 hours after the surgery.[4] Significant decline of IL-6 is observed on post-operative day 1 and it returns to near pre-operative level on post-operative day 2.[5,6] In one study, IL-6 level of more than 432 pg/ml on post-operative day 1 is associated with higher risk of complication, and longer hospital stay.[7]

CRP level on the other hand, peaks at 48 hours after surgery as described in most studies.[7-11] In an analysis of 1187 patients who underwent colorectal cancer surgery, Warschkow et al revealed that median level of post-operative CRP in the absence of inflammatory complications are 94.5 mg/L on Day 1, 142.0 mg/L on Day 2, 123.0 mg/L on Day 3, 78.0 mg/L on Day 4, and 56.5 mg/L on Day 5. In those with post-operative inflammatory complications such as anastomotic leak, the CRP value continue to rise on day 3 after surgery. They concluded that CRP level of 123 mg/L on post-operative day 4 is a statistically optimal cut-off value for CRP to discriminate between patients with and

without inflammatory complications.[9] Some other studies are in tune with this concept that CRP level beyond 100 mg/L on post-operative day 4 should warrant the clinician to consider anastomotic dehiscence in cases where gastrointestinal anastomosis are constructed.[8,10,11] While most studies revealed that CRP level on day 4 are the most reliable for suspicion of inflammatory complications, Rettig et al on the other hand, demonstrated that a CRP level of 203 mg/L on post-operative day 3 would be discriminative enough for increased risk of complications.[7]

The stress responses following surgery can be attenuated and therefore, recovery can be enhanced by multimodal clinical strategies. Surgical Nutrition is one of the key aspects in peri-operative management that is able modulate body response to the surgical stress in addition to optimal analgesia, neural blockade, peri-operative fluid management and laparoscopic surgery. In fact, these have been among the components of Enhanced Recovery After Surgery (ERAS) Protocol. Pre-operative carbohydrate loading, in addition to early resumption of post operative oral feeding have been proven to accelerate recovery and have great impact on hospital stay and overall hospital costs.[12]

More and more emerging evidence support the safety of early oral nutrition post operatively. Even in the presence of bowel anastomosis, early oral nutrition is still regarded as safe and does not significantly contribute to anastomotic breakdown.[13,14] Several studies have also reported that early oral feeding promotes early return of bowel function, which leads to shorter hospital stay.[13-17] Delay in resumption of feeding post operatively is associated with more infectious complications and delayed recovery.[18-20] Early oral diet is safe 4 hours after surgery in patients with a new non-diverted colorectal anastomosis.[21] Early normal food or enteral nutrition including clear liquids on the first or second post-operative day does not cause impairment of healing of anastomoses in colon and rectum. As spontaneous oral intake and the degree of tolerance would vary

among individuals post-operatively, the type of diets, i.e., liquid or solid diet, is decided according to the clinical situation. This would take into consideration of the types of surgery performed.[23] The use of Oral Nutrition Supplement (ONS) can be considered as an adjunct to match total daily calorie requirements.[21-23] The enteral delivery of nutrition has been reported to improve glycaemia by enhancing insulin secretion.[20] Its mechanism has yet to be elucidated but it is thought to be related to the physiological effect of utilising entero-pancreatico-hepatic axis.[24] It is also reported that it triggers the release of gut peptides such as Incretin and glucose-dependent insulinotropic peptide which stimulate insulin release and inhibit glucagon secretion. It also preserves gut mucosa and stimulates secretion of immunoglobulin A which reduces bacterial translocation and overgrowth.[25,26]

Kelulut Honey is a type of honey produced by Stingless Bee (Meliponine sp). This type of honey is found in tropical and sub-tropical regions and it has sweet-sour taste with minimal crystallisation. From nutritional aspect, it mainly consists of fructose and glucose.[27-29] Recently it is found that *Kelulut* honey is a novel source of trehalulose, an isomer of sucrose with an unusual glucose-fructose linkage. Trehalulose is known to be acariogenic and it has low glycemic index. For each 100 g of *Kelulut* honey, it contains 13 - 44 g of trehalulose.[30] *Kelulut* Honey is also known for its higher proportion of polyphenols when compared to other types of honey. This contributes to its anti-inflammatory and anti-oxidant properties, and this proves to be beneficial for wound healing.[31,32] *Kelulut* Honey also contains small amounts of proteins, amino acids, enzymes, vitamin and mineral. Anti-bacterial properties of honey including *Kelulut* Honey was also reported.[33]

The use of *Kelulut* honey in diabetic individual would trigger concern on its effect on provoking hyperglycaemia. However, *Kelulut* Honey possesses low-glycaemic index and would not trigger rapid elevation of blood sugar in diabetics. This is especially true

when we consider its trehalulose content.[30] Honey in general, is reported to cause lesser elevation of plasma glucose level, insulin, C-Peptide and CRP when compared with dextrose.[34] A quasi-experimental intervention study among 60 adult patients with impaired glucose tolerance concludes that daily intake of 30 g *Kelulut* Honey for a month resulted in insignificant effect on fasting blood glucose, fasting lipid profiles, and other metabolic parameters.[35]

With extensive academic research on *Kelulut* Honey, hygienic and medically safe *Kelulut* Honey production are made possible. The MUSTAFA-Hive (Meliponiculture Using Split-able Throne within Air-jacketed Palace For Amplification-Hive) has facilitated colony expansion as well as effectively promotes a more hygienic honey production. As Malaysia's tropical climate in addition to its high humidity level result in honey with high moisture contents that causes fermentation, an apparatus called HILDA (Honey Interlinked Dehydration and Dispenser Apparatus) was invented. This has allowed *Kelulut* Honey dehydration without heating from 35% to around 18%, and therefore prevent fermentation and contamination.[28] It also raises the quality of the honey. Introduction of MS2683:2017 is a cornerstone in ensuring the quality and safety of the *Kelulut* Honey.[36]

Considering both metabolic and anti-inflammatory benefits of *Kelulut* Honey reported in many studies, as well an established method and standard in its production, we propose a clinical study to evaluate its benefits in surgical patients. This study is designed to evaluate the effect of *Kelulut* Honey on clinical and immunological response in patients undergoing lower gastrointestinal surgeries.

2.2. PROBLEM STATEMENT & STUDY RATIONALE

Nutrition is among the major aspect influencing post-operative recovery, and therefore post-operative oral feeding must be established once it is safe to do so. Delivery of the nutrition may start with simple diet, usually a clear fluid regimen, which is then stepped up until solid diet is fully established one or two days later. The calorie supplied is initially below the actual energy expenditure, and over the next day or two, it is gradually increased in accordance with the degree of tolerance to the diet until it provides more than 80% of estimated total energy requirements.[21,23] This measure is vital in modulating the post-operative metabolic, immunologic and inflammatory response which in turn, enhances post-operative recovery. Early feeding has been shown to promote earlier return of bowel function and reduces the risk of post-operative ileus.

Despite the intention of modulating post-operative stress response via early introduction of oral feeding, the implementation might prove to be troublesome at times. It is recommended that intake should be at least 50% of total daily requirement, and in those who cannot achieve this minimal target beyond 5 days should have a nutrition therapy started, enterally or parenterally. To date, there is no standard diet model to guide the commencement of post-operative diet to achieve this recommended target caloric value which in turn will improve overall post-operative stress response. It is generally accepted that oral diet would begin with introduction of liquid diet in the form of clear fluid, but the understanding of this concept differs among clinicians and patients. There is a general tendency to equate clear fluid to merely plain water, while its actual definition is a liquid which contains easily digested molecules, leaving no undigested residue in gastrointestinal tract. Provision of such fluid also usually lacks monitoring in terms of the volume consumed as well as its estimated calories. The suggested clear fluid tends to be

those which are commercially available in the hospital mart. However, these are either containing too little energy, or are those of high glycaemic index which are not suitable for diabetics. There are some clear fluids or milk-based diets which are commercially manufactured for the purpose of post-operative nutrition, for example, Carborie® by Valens. However, these products are expensive and may have serious implication on healthcare budget if the purchase are made routine for every post-operative patient.

For these reasons, we are turning into *Kelulut* Honey. It is a natural product which is known for its antioxidant and anti-inflammatory properties, as well as its nutritional value. It has low glycaemic index and therefore is safe in diabetics. As it contains the simplest form of carbohydrate, it would serve as a clear fluid in post-operative patients. This would serve as a 'bridge' to semi-solid and solid diet post-operatively. Its antioxidant and anti-inflammatory effects would provide added benefits to post-operative patients. An improved post-operative stress response would shorten the ileus and therefore promotes earlier return of bowel function.

These effects of *Kelulut* Honey have yet to be evaluated clinically. We propose a clinical study to evaluate the effect of *Kelulut* Honey on clinical, inflammatory and immunological response in patients undergoing lower gastrointestinal surgeries.

2.3. RESEARCH QUESTION(S)

1. Does the use of *Kelulut* Honey given post-operatively facilitate earlier return of Gastrointestinal function?
2. Does *Kelulut* Honey significantly improve post-operative inflammatory and immunological markers and therefore reduce risk of post-operative complications?

2.4. OBJECTIVES

To determine the effect of *Kelulut* Honey consumed in the early post-operative period on the return of bowel functions and on the post-operative inflammatory and immunological markers in patients undergoing lower gastrointestinal surgery.

Specific Objectives:

1. To compare the time of the first flatus and first bowel evacuation between *Kelulut* Honey and Carborie® when consumed in the early post-operative period after lower gastrointestinal surgery.
2. To associate the change of C-Reactive Protein (CRP) and Interleukin-6 (IL-6) pre and post-operatively in patients consuming *Kelulut* Honey versus Carborie® in the early post-operative period after lower gastrointestinal surgery.

2.5. RESEARCH HYPOTHESIS

First Hypothesis

H_0 : There is no significant difference between the time of the first flatus and first bowel evacuation between *Kelulut* Honey and Carborie® when consumed in the early post-operative period after lower gastrointestinal surgery.

H_1 : There is a significant difference between the time of the first flatus and first bowel evacuation between *Kelulut* Honey and Carborie® when consumed in the early post-operative period after lower gastrointestinal surgery.

Second Hypothesis

H_0: There is no significant difference between the level of C-Reactive Protein (CRP) and Interleukin-6 (IL-6) pre- and post-operatively in patients consuming *Kelulut* Honey versus Carborie® in the early post-operative period after lower gastrointestinal surgery.

H_1: There is a significant difference between C-Reactive Protein (CRP) and Interleukin-6 (IL-6) pre- and post-operatively in patients consuming *Kelulut* Honey versus Carborie® in the early post-operative period after lower gastrointestinal surgery.

2.6. CONCEPTUAL FRAMEWORK

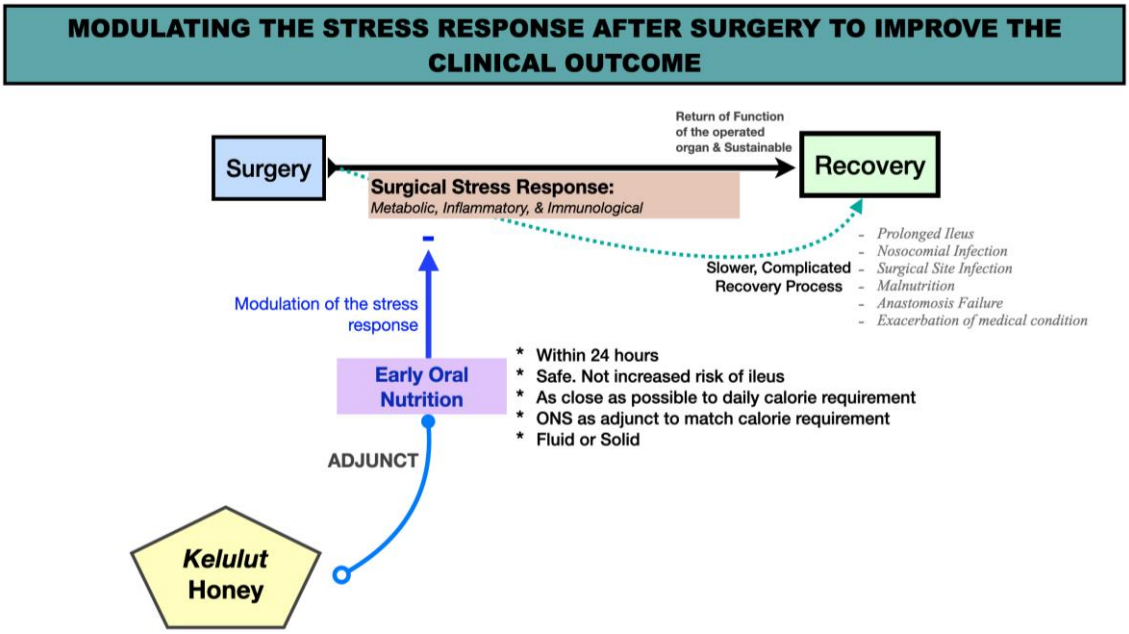

Figure 1: Modulation of stress response after surgery

2.7. RESEARCH DESIGN

This is a single-centre, double-blinded, randomised controlled trial. This trial will be registered in www.clinicaltrials.gov

2.8. STUDY AREA

This study will be performed in Hospital Universiti Sains Malaysia (Hospital USM), Kubang Kerian, Kelantan, Malaysia.

2.9. STUDY POPULATION

Patients undergoing surgery for lower gastrointestinal tract in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

2.10. SUBJECT CRITERIA

Inclusion Criteria:

1. Age is at least 18 years old
2. Open or laparoscopic small bowel resection and anastomosis.
3. Open or laparoscopic large bowel resection and anastomosis, with or without covering stoma.
4. Open or laparoscopic anterior resection with or without covering stoma
5. Open or Laparoscopic abdomino-perineal resection.
6. Repair of Irreducible Inguinal hernia that involves resection of small or large bowel.
7. Laparotomy with extensive small or large bowel adhesiolysis.

Exclusion Criteria:

1. Nutritional Risk Screening (NRS 2002) Score of 3 or more

2. American Society of Anaesthesiologists (ASA) Classification of 4 or more.
3. On long-term regular steroid therapy
4. Known allergy to honey or maltodextrin
5. On fluid restriction
6. Sepsis on admission

Withdrawal criteria:

1. Refusal or change of mind

2.11. SAMPLE SIZE ESTIMATION

Calculation of the sample size was made for each objective to match a power of 80%, with significance level (α) set at 0.05. Both study arms will have equal number of samples.

For objective 1, the calculation of sample size was based on recent almost similar study conducted by Nematihonar, B., et al (2019).[20] Manually calculated pooled standard deviation between experimental and control groups yields 15 hours for time of first bowel evacuation after small bowel anastomosis. Using this information, sample size was calculated using G*Power Software. A 10-hour difference between two groups would be be significant, and therefore the effect size is 0.667. This yields a sample size of 37 participants in each group. With 10% dropout is expected, the sample size is 42 participants per group. (Total = 84 Samples).

For objective 2, G*Power software was also used to calculate the sample size. Effect size of 0.8 was chosen, and this calculation yields 26 participants per group. After 10% dropout is included, the sample size is 29 participants. Finally, for this study, the larger sample size is chosen. With 10% of dropout is anticipated, the final sample size will be 84.

2.12. SUBJECT RECRUITMENT, RANDOMISATION AND MASKING

Patients who are admitted for lower gastrointestinal tract surgeries are eligible to participate in this study. After those who are ineligible are excluded, patient who consented to be in this study will be consecutively assigned to a number, from 1 to 84.

Randomisation into two equal size groups, i.e., the experimental (*Kelulut* Honey) and control (Carborie®) groups, will be carried out by a statistician who is not involved in the recruitment process, assessment and treatment of the patients. Random sequence will be generated by Permuted Block Randomisation with multiple Random Block size method. 84 random sequences will be generated prior to the recruitment of participants.

Blinding will involve the clinicians, the operating surgeons, anaesthetists, nurses, and the outcome assessors. Blinding will continue until the period of data analysis which will be carried out after completion of data collection. This will be made possible by Dr Zulkifli and his team who will be preparing both types of the beverages, according to the randomly generated sequence. Dr Zulkifli and his team will not be involved in the recruitment, treatment and outcome assessment of the patients. Each serving of the beverage will be prepared in an unlabelled bottle which contains either *Kelulut* Honey or Carborie®. The beverages will be stored in a fridge prior to dispensing to the patient. Each serving contains 500 ml of the beverage, and participants are required to consume them within 5 hours. The beverage will be served 3 times a day at regular interval, and therefore total of 1.5 L of the beverage (*Kelulut* Honey or Carborie®) will be given daily to each participant.

Each 500 ml-serving contains 235 Kcal of either *Kelulut* Honey or Carborie®. With 3 servings of the beverage, each participant will receive 706 Kcal per day. This is equivalent to 40% of total calorie requirement of 25 kcal/kg/day of a 70-kg individual.

Malaysian Clinical Practice Guidelines (CPG) on Diabetes Mellitus recommends that at least 130 g of carbohydrate (equivalent to around 520 Kcal or around 30% of daily calorie) is required daily to prevent ketosis.[37] In addition, numerous low-carbohydrate diet in diabetics considered 40% carbohydrate daily as the upper limit of this type of diet intervention.[38-40] Our study adapts this concept since carbohydrate is the main constituent of *Kelulut Honey.* By setting 70-kg as standard body weight of our population, 40% of daily energy requirement gives rise to 706 kcal/day. This would translate into 231 g of *Kelulut* Honey per day, or 186 g of Carborie® per day.

The *Kelulut* Honey used in this research is a medical-grade Kelulut Honey which has undergone dehydration without heating down to 18-20%, and therefore contamination and fermentation can be prevented. A serving of *Kelulut* honey contains 77 g (=235 Kcal) of such honey and is diluted with 500 ml of water prior to the consumption. On the other hand, a serving of Carborie® used in control group contains 62 g (=235 Kcal) of maltodextrin and is also diluted with 500 ml of water before consumption. In this study, participants are required to consume this beverage for 2 days; On post-operative days 1 and 2. Nutritional Information of Kelulut Honey and Carborie are as described in the following tables.

Table 1, Nutritional Information of Kelulut Honey-1

TEST PARAMETER	UNIT	TEST METHOD	RESULT
Calories	Kcal/100g	By calculation (based on Method of Analysis for Nutritional Labelling, AOAC, 1993, PAGE 106 & 5)	306
Carbohydrate	g/100 g	By calculation (based on Method of Analysis for Nutritional Labelling, AOAC, 1993)	75.2

Table 1, Nutritional Information of Kelulut Honey-1

TEST PARAMETER	UNIT	TEST METHOD	RESULT
Sugar	g/100 g	In-house method, CL-TM-01-006, based on AOAC 974.06 & AOAC 925.05, 2005; The Chemical Analysis of Food by Pearson, 7th Ed (Lane-Eynon Titrimetric)	55.6
Dietary Fibre	g/100 g	AOAC 985.29, 2005	0.9
Protein	g/100 g	In-house method, CL-TM-01-018, based on AACC 46-12, Vol II 9th Edition	1.2
Total Fat	g/100 g	AOAC 950.54	N.D (<0.1)
Calories from fat	Kcal/100g	By calculation (based on Method of Analysis for Nutritional Labelling, AOAC, 1993)	N.D (<1)
Saturated fat	g/100 g	In-house method, CL-TM-01-011, based on AOCS Ce-2-66, 2005 & AOCS 1d-91, 2005 (GC)	N.D (<0.01)
Cholesterol	mg/100g	In-house SGS/SOP/FL/Chem/015 Based on AOAC 994.10 (GC-FID)	N.D (<0.30)
Trans Fat	g/100 g	In-house method, CL-TM-01-001, based on AOCS Ce1c-89, 2005 (GC)	N.D (<0.01)
Sodium	mg/100g	In-house method, CL-TM-01-020, based on AOAC 985.01 & AOAC 922.02.2005; AACC 40-70, Vol II 9th Ed (ICP-OES)	2.32
Calcium	mg/100g	In-house method, CL-TM-01-020, based on AOAC 985.01 & AOAC 922.02.2005; AACC 40-70, Vol II 9th Ed (ICP-OES)	27.80
Iron	mg/100g	In-house method, CL-TM-01-020, based on AOAC 985.01 & AOAC 922.02. 2005; AACC 40-70, Vol II 9th Ed (ICP-OES)	0.08