Small bowel responses to enteral honey and glutamine administration following massive small bowel resection in rabbit.

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Summary
The trophic effects of honey and glutamine in the healing and adaptation of the small bowel following intestinal resection were studied in some Nigerian non-descript breeds of rabbits. Nine rabbits of mixed sexes with mean body weight of 1.45 ±0.55kg were used. They were randomised into three treatment groups following 50% small bowel resection. Group A rabbits were placed on oral honey treatment, Group B on oral glutamine and group C on normal saline (control). All groups exhibited signs of small bowel adaptation (glutamine(B) honey (A) control (C) at the end of the experiment (4 weeks) with oral glutamine showing the best overall effects on intestinal mucosal growth and adaptation evidenced by significant increase (P<0.05) in residual bowel length (37.3%), villi width (20.0%), crypt depth (113.3%) and a non significant increase in villus height (33.3%) and cellular mass (10.2%). Honey showed a better effect than control with a significant increase (P<0.05) in villi width (18.2%), crypts depth (66.7%) and cellular mass (33.9%) and a non significant increase in gross residual bowel length (24.6%), and villus height (30.5%). Our result shows that honey and glutamine have trophic effects on bowel mucosa healing and hyperplasia and have potential therapeutic effects on massive bowel resection in humans.

Keywords: *Small, bowel, honey, glutamine, massive, resection*

Résumé
Les effets trophiques du miel et de la glutamine dans la guérrison et l'adaptation de petit ulcéres aprés la resection intestinale étaient étudiés chez certains lapins d'espéces Nigérian. Neuf lapins de sexe different ayant un poids moyen de 1.45 ± 0.55 kg étaient utilisés. Ils étaient divisés au harzard dans trois groupes en fonction du dégré de resection ou ulcération de l'estomac. Les lapins du groupe A recevaient du miel oralement pour le traitement, ceux du groupe B recevaient la glutuamine et ceux du groupe C servant comme le contrôle ne recevaient de l'eau salée uniquement. Tous les groupes demontraient des signes d'adaptation á l'ulcération á la fin des quatres semaines d'experiences. L'administration orale du glutamine avait le meileur effet sur le developpment des mucoses intestinales, une adaptation significative (P<0.05) et une croissance de la longeur de l'estomac résiduel(37.3%), de la largeur des villi (20.0%), de la hauteur des cryptes(113.3%) et une augmentation non significative de la hauteur des villi(33.3%) et de la masse cellulaire(10.2%).Le meil demonstrait un effet meilleur que la solution salée administrée aux controles avec une augmentation significative de la largeur des villi (18.2%) (P<0.05), de la profondeur des cryptes(66.7%), de la masse cellulaire de 33.9% et une augmentation non significative de la longeur de l'estomac résiduel de 24.6% et l'hauteur de villi de 30.5%. Nos résultats montrent que le miel et la glutamine ont des effets trophiques sur le traitement des mucoses de l'estomac et l'hyperplasie, et ont des effets thérapeutiques potentiel sur la resection massive de l'estomac chez l'homme.

Introduction

The gastrointestinal tract functions include the digestion and absorption of nutrients into the body system. Any structural and, or functional disorder of the system may require medical attention, or surgical intervention. Surgical intervention in disease conditions of the intestine (resection and anastomosis) is indicated in cases of congenital anomalies, neoplasia, inflammatory bowel disease, and the relief of mechanical obstruction (intussusception, volvulus, torsion) [1,2]

Intestinal resection, when massive (fifty percent and above) results in serious nutritional consequences in man, dogs, cats, and rodents [3,4,2,1]. The goal of management of the consequent clinical challenge is focused at treating the post-operative manifestations associated with Short Bowel Syndrome (SBS) and facilitating adaptation; which

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is a sequence of changes following bowel resection to improve the functional integrity of the residual gut and prevent gastrointestinal insufficiency [4,5]. Post resection adaptive hyperplasia of the small bowel has been known to be influenced by a complex variety of factors such as gut hormones, digestive enzymes, length of residual bowel and presence or absence of ileocolic valve [6].

Over the last three decades, patients with massive intestinal resection have had a greater chance of survival with intravenous nutrient packs referred to as Total Parenteral Nutrition (TPN). TPN however is associated with multiple complications. Apart from the high cost involved, TPN does not promote the adaptation of the residual bowel segment [7,2].

A major focus of research, therefore, has been to identify trophic substances that could enhance rapid adaptation of the residual bowel, and allow maximum enteral autonomy. In recent years, pharmacological products, such as, growth hormones, glutamine and other anabolic substances have been suggested but with conflicting results from experimental trials [2]. Glutamine supplementation, however, has been confirmed to be of benefit to SBS patients as a key component in the maintenance of healthy intestinal mucosa [8].

Honey is a natural product with many medicinal uses as described by traditional medicine [9,10,2]. Honey is effective in stimulating the growth of new tissues in the repair process associated with damaged intestinal mucosa [11]. There is, however, a dearth of information in literature on the use of honey as a therapy for patients' who had undergone bowel resection. The aim of this study is to examine and compare the responses of the residual small bowel (following massive resection) to honey and glutamine therapy using the rabbit as experimental model.

Materials and methods

Experimental animals

Nine (9) non- descript local rabbits of either sexes (4 intact bucks, 5 non- pregnant and non- lactating does) with mean body weight of 1.45kg ± 0.55 SD were studied. They were sourced from local breeding units in Ibadan, Nigeria, and housed in the experimental animal unit, in individual cages that provided ample space for exercise. They were provided with commercial rabbit feed (Guinea feed Nig. plc), and water ad libitum. The rabbits were conditioned for three weeks and judged to be of good general health based on complete physical examinations before the commencement of the experiment.

Anaesthesia

Each animal was taken off feed 2 hours prior to surgery but had access to water ad-libitum. Premedication was done with Pentazocine® (Fortwin, Ranbaxy pharmaceuticals Ltd, India) at the dose rate of 5mg/kg and Xylazine® (Kepro, Holland) at the dose rate of 3mg/kg. Surgical anaesthesia was achieved with Ketamine hydrochloride (50mg/ml) (Rotex medica, Trittau, Germany) administered at the dose rate of 35mg/kg. All the drugs were injected through the quadriceps group of muscles.

Experimental design and surgical procedure

The ventral abdomen was aseptically prepared, and the intestine was approached through a ventral midline abdominal incision. The intestinal loops were exteriorized over moist laparotomy sponges. The Treitz ligament was located and the small bowel length was determined as earlier described by Eyarefe *et al* [1]. Fifty percent (50%) of the small bowel length from 5cm distal to the Treitz ligament was resected. Using Polyglactin 910 (Vicryl® Ethicon, USA), the residual bowel segment was apposed with an end to end anastomosis as earlier described [12]. The laparotomy incision was closed using standard surgical technique. A 3cm full thickness biopsy from sectioned segment was fixed in 10% formalin (Sample A). Animals were placed in recovery cages and monitored for 24 hours before being returned to the experimental animal cages. They were restricted to water on the 1st post operative day and feeding was commenced on the 2nd post-operative day.

Following resection, the rabbits were randomised into three treatment groups (A, B and C). Group A rabbits (n=3) were placed on 0.15kg commercial rabbit feed (Guinea feeds Nig. plc) mixed with honey. The quantity of honey was gradually increase from 1-3ml. Water was provided ad libitum. Group B rabbits (n-3) were placed on 0.15kg feed mixed with glutamine powder (2g/kg body weight per day). Water was provided ad libitum. Group C rabbits (n-3) were placed on 0.15kg feed and water ad libitum (control group). After the treatment duration (4 weeks), the animals were sacrificed, and the residual bowel segment of each animal was examined grossly and the length determined. A 3cm full thickness biopsy section was obtained from residual bowel segments (Sample B) and fixed in 10% formalin.

Histomorphometric evaluation

A paraffin section of tissue samples was sliced at a thickness of 4µm and stained with haematoxylin and

eosin. Measurements of mucosal villus height and crypt depth were taken using a micrometer rule, as described by Joaquim *et al* [13]. The number of cells was estimated by counting the epithelial cell nuclei for each villus. For each parameter, at least 3 readings were taken from different villi and an average value calculated.

Statistical analysis

The results are presented as mean ± SD. Post hoc comparison of pairs of means was performed using one way Analysis of Variance (ANOVA) and Student's t–test. The level of significance was set at 0.05.

Result

The effects of the treatment on various parameters are as follows:

Faecal consistencies

Seven out of the nine rabbits (78%) in all the three groups passed out brown pasty faeces as opposed to the normal pelleted faecal droppings of rabbits from the 1st to 3rd post operative day. Fecal dropping became normal thereafter.

Feed intake

All rabbits showed an initial decrease in appetite, followed by a steady increase over 4 weeks. No group, however, returned to the quantity of feed (0.15kg) consumed daily before the resection. Group A (honey treatment) had an average feed consumption of 0.10kg while group B (glutamine treatment) 0.05kg and group C (control) 0.05kg.

Table 1: Body weight changes (kg) in the rabbits within two weeks post resection

Day	Honey	Glutamine	Control
0	1.83±0.10	1.50±0.5	1.27±0.15
2	1.73±0.03	1.43±0.5	1.13±0.18
4	1.63±0.08	1.38±0.5	1.13±0.18
6	1.68±0.03	1.45±0.5	1.10±0.15
8	1.63±0.03	1.43±0.5	1.15±0.15
10	1.68±0.03	1.43±0.5	1.15±0.15
12	1.70±0.05	1.45±0.5	1.20±0.15
14	1.78±0.08	1.48±0.5	1.23±0.19

Group A = Honey treatment
Group B = Glutamine treatment
Group C = Control (Normal saline)

Body weight changes

There was a rapid fall in weight in all the three groups within the first two weeks (Table1), with the lowest weights in each group recorded between days 4 and 6. After this initial decline in body weight all the rabbits showed a gradual and steady increase in weight. At 2 weeks, the average weight in group A was 0.05kg less than the pre operative weight, the average weight in group B was 0.02kg less than the pre operative weight while the average weight in group C was 0.04kg less than the pre operative weight. However, at 4 weeks, there was an observed increase in weight above the pre operative weight, the control group having an average increase of 0.01kg and the group on honey with an increase of 0.02kg. There was no significant difference in the body weight changes in the three groups ($p>0.05$)

Table 2: Changes in residual bowel length (mm) four weeks post resection

Groups	Honey	Glutamine	Control
a	77.75±11.25	79.75±7.25	98.0±6.25
b	96.85± 5.15	109.50±2.50	117.0±3.55
c	19.10± 6.10	29.75±9.75	19.7±4.75
% increase	(24.6%)	(37.3%)	(20.1%)

a = Measured residual length after resection
b = Measured residual length after treatment period
c = Increase in length

Residual small intestinal length

There was an increase in length of the residual small intestine in the three groups (Table 2).The rabbits on glutamine treatment had a significant ($P<0.05$) highest increase 37.3% in residual length, followed by the group on honey with a non significant increase ($p>0.05$) of 24.6% and the control group 20.1%.

Table 3: Changes in villus height (mm) of the residual bowel four weeks post resection

Groups	Honey	Glutamine	Control
A*	0.59±0.03	0.51±0.16	0.54±0.01
B*	0.77±0.04	0.68±0.99	0.69±0.01
% increase	(30.5%)	(33.3%)	(27.8%)

A = measurements of samples taken after resection*
B = measurement of samples taken after the treatment period*

Villus height

All the three groups showed non significant increase in villus height ($p>0.05$) after the treatment period (Table3). The greatest increase in height (33.3%)

was shown by the group on glutamine with the group on honey having an increase of 30.5%. The control group had the lowest increase in villus height (27.8%).

Villus width

There was a significant ($P<0.05$) decrease in villus width among the three groups (Table 4). The highest decrease (20%) was shown by the group on glutamine, followed by the group on honey (18.2%). The least amount of decrease was from the control group (11.1%).

Table 4: Changes in villus width (mm) of the residual bowel four weeks post resection

Groups	Honey	Glutamine	Control
A*	0.11±0.2	0.10±0.1	0.09±0.1
B*	0.09±0.2	0.08±0.1	0.08±0.1
% decrease	(18.2%)	(20.0%)	(11.1%)

A = measurements of samples taken after resection*
B = measurement of samples taken after the treatment period*

Table 5: Changes in crypt depth (mm) of the residual bowel four weeks post resection

	Honey	Glutamine	Control
A*	0.03±0.002	0.03±0.003	0.03±0.002
B*	0.05±0.003	0.07±0.001	0.04±0.002
% increase	(66.7%)	(113.3%)	(33.3%)

A = measurements of samples taken after resection*
B = measurement of samples taken after the treatment period*

Table 6: Changes in cellular mass (average number of cells/villus) of the residual bowel four weeks post resection.

	Honey	Glutamine	Control
A*	118±49	127±39	110±16
B*	158±40	140±29	119±6
% increase	(33.3%)	(10.2%)	(8.2%)

A = measurements of samples taken after resection*
B = measurement of samples taken after the treatment period*

Crypt depth

There was a significant increase ($P<0.05$) in crypt depth of glutamine and honey group when compared with control (Table5). The group on glutamine showed a very high increase of 113.3%, followed by the group on honey, 66.7%, and then the control group, 33.3%.

Cellular mass

There was a significant increase ($P<0.05$) in the average number of enterocytes per villus of the honey treated group when compared with those of glutamine and control in all the three groups (Table 6). Honey treatment had the highest cellular increase (33.9%), followed by glutamine treatment (10.2%) and the control (8.2%).

Discussion

The result of this study shows that both glutamine and honey have beneficial effects in the process of intestinal adaptation following massive resection, with glutamine showing a better trophic effect. The gradual increase in the volume of honey given to each rabbit (1-3ml) was done to reduce the predicted diarrhoea due to intestinal hurry precipitated by the shorter gut. Examination of the anastomotic site in the three groups following euthanasia, showed evidence of healing without signs of leakage, stricture, scaring, adhesion, torsion, obstruction or peritoneal infection. The passing of diarrheic faeces among the three groups between 1st to 3rd post operative days was consistent with findings in similar studies [14,1]. Resolution of the diarrhoea by the third post operative day was an indication that adaptive changes had begun [5]. Adaptive changes begin 12 -24 hours after massive intestinal resection and will continue for more than a year after resection, and with bowel compensation, diarrhoea and malabsorption are reduced [14,5]. The initial weight loss observed in this study was consistent with those observed by other investigators [13]. Clinical weight loss was defined as loss of up to 10% of the total body weight [15]. The fall in weight may be due to catabolic weight loss from surgical stress and wound healing [1]. It could also be attributed to the reduced capability of the residual bowel to digest and absorb nutrients at the early post operative period [16]. The later gain in body weight experienced by the honey group may be due to the appetite boosting effect of honey and improved digestion and absorption following intestinal adaptation. Oral glutamine showed the best overall effect on intestinal mucosal growth and adaptation in this study evidenced by significant increase ($P<0.05$) residual bowel, villi width (20.0%), crypt depth (113.3%) and a non significant increase in cellular mass (10.2%) and villus height (33.3%). The rabbits treated with glutamine had the highest decrease in villus width signifying that they had the

highest number of intestinal villi per mucosal area. Also there was greater intestinal glandular activity shown by increase in crypt depth (113.3%) leading to more digestion and absorption of feed nutrients. Honey, however, had a good trophic effect on intestinal healing and adaptation as oral glutamine evidenced by a significant increase in villus width (18.2%), cellular mass (33.9%), and mucosa glandular activity (66.7%) and a non significant increase in residual bowel length (30.5%), and villus height (30.5%).

The gain in residual intestinal length, villus height, crypt depth, and cellular mass in animals fed with glutamine and honey compared with control confirms the benefit of both substances in enhancing and maintaining healthy intestinal mucosa [9,17,11]. The benefit of glutamine therapy in management of massive intestinal resected patients has been well studied [17,18]. Glutamine rather than glucose is the major fuel for intestinal metabolic functions [19] and it is a key player in maintaining the immune status of the bowel [20]. In the current study, we observed an increase in the demand for glutamine by the animal body after massive resection. We then postulate that high concentration of enteral glutamine will be required by the patient to achieve better intestinal adaptive results. The relatively low concentrations (4% and 5%) of enteral glutamine used by some investigators may be responsible for the inconsistencies in the result they had in similar studies [21]. The early post operative management of the resulting complication is important in the survival of the patient, and restoration of enteral autonomy. Honey had good trophic effects on the residual bowel following resection comparable to glutamine. Since it is more available and relatively cheaper it could be recommended for the management of massive intestinal resected patients especially in developing countries. The mechanism by which glutamine benefits the bowel following massive resection has been studied [22,17]. More work is therefore required to investigate the mechanisms by which honey exerts its trophic effects on the intestine following massive intestinal resection.

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