Niger. J. Physiol. Sci. 27(December 2012) 189 –193
www.njps.com.ng

Niger. J. Physiol. Sci.

Effects of honey, glutamine and their combination on canine small bowel epithelial cell proliferation following massive resection

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Summary: The effects of honey, glutamine and honey/glutamine combination on the healing and adaptive process of the bowel following massive small bowel resection were studied in some Nigerian non-descript breeds of dogs. 24 dogs (3-4months old) of mixed sexes with mean body weight of 4.42±0.70 kg were studied. They were randomized into four treatment groups following 70% small bowel resection. Group A dogs were placed on glutamine treatment, Group B on oral glutamine/honey and group C on honey and group D normal saline (control). Their body weights were evaluated for 15 days and the pre- and post-treatment gut biopsy samples were obtained and processed for morphometric evaluation. All groups exhibited signs of small bowel adaptation (Glutamine/honey>glutamine > honey > control) at the end of the experiment (4 weeks). Glutamine/honey combination, glutamine and honey had gradual increase in body weight from days 3-15 of weight evaluation. The control group, however, had a remarkable drop in body weight compared with other groups. Oral glutamine/honey combination showed the best overall effect based on body weight gain, intestinal mucosal growth and adaptation, evidenced by increased in residual bowel Villi height (27.71µm), Villi weight (14.51µm), Crypt depth (11.25µm), and Villi density (3.40µm). Glutamine showed a better result than honey with a significant increase in villi height (38.08µm), width (8.48µm) and crypt depth (40µm). Histologically, an improved villi branching was observed with glutamine/honey combination. Our results showed that honey/glutamine combination had comparative therapeutic advantage over glutamine or honey and may be a preferred treatment for short bowel syndrome patients.

Keywords: Bowel, Epithelial cell, Proliferation, Honey, Glutamine.

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Manuscript Accepted: December,2012

INTRODUCTION

The clinical management of Short Bowel Syndrome (SBS) sequel to massive bowel resection, which is characterized by chronic maldigestion, malabsorption and diarrhea has been a challenge to most gastroenterologists (Eyarefe et al., 2001, AGA, 2003, Brown and Dibaise, 2004, Gorman et al., 2006, Eyarefe et al., 2008). Management of this clinical challenge (SBS) has been a major task compelling a multi-disciplinary therapeutic approach and research efforts focused at promoting small bowel adaptation: which is a series of gradual gross and histological changes within the bowel wall and mucosal glandular epithelium to compensate for the shortened bowel length and to improve the functional integrity of the residual gut, thus preventing gastrointestinal insufficiency (Brown and Dibaise, 2004; Eyarefe et al., 2008) and besides, the cost of promoting these adaptive changes are enormous. A recent retrospective cohort study in Canada (Navarro et al., 2009) estimated the incidence of SBS to be 24.5 per 100,000 live births with an associated mortality rate of 38%, (Wales et al., 2004). Mortality rates and costs in Europe are comparable to those in North America. In Graz, Austria, the overall mortality in infants who had SBS ranged from 15% to 25% and the annual cost per patient was $100,000 to $150,000 (United State Dollars) (Schalamon et al, 2003). Although, data from Africa and Asia are not available, they may be predictably higher.

A major research need is identification of substances that could enhance a rapid regeneration of bowel epithelial cells, induce enlargement of the muscular wall, promote nutrient absorption and allow for 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 clinical trials in human and animal species (Brown and Dibaise, 2004). Enteral or parenteral glutamine supplementation, however, has been reported to be of benefit to SBS patients, as an important agent in the maintenance of healthy intestinal mucosa (Atkins, 1998). However, the cost and availability has been the limiting factors affecting glutamine utilization especially in third world countries. Also, Total Parenteral Nutrition (TPN) although lifesaving in the immediate period, is expensive, and long term dependence is associated with complications such as; intestinal atrophy, electrolyte and metabolic alterations, increase intestinal permeability, sepsis, cholestasis with hepatic and pancreatic failure; besides, TPN does not promote the adaptation of the residual bowel (Vanderhoof and Langnas, 1997, Sawyer et al., 2004). There is therefore a need for a substance that is readily available, affordable and capable of enhancing bowel adaptation without complication as this is critical to SBS management.

Honey is a natural product that has gained popularity, in recent times in therapeutic medicine. Its effects in the treatment of different types of wounds (Molan, 1999) and in the management of upper gastrointestinal lesions have been documented (Haffejee and Moosa, 1985; Obi *et al.*, 1994, Swayeh and Ali, 1998,)**.** There is however, a dearth of data on the use of honey in promoting the gut adaptive process following massive small bowel resection. Moreover, the comparative advantage of honey/Glutamine over glutamine or honey in the gut adaptive process has not been reported in literature. Our preliminary findings on the effects of honey and glutamine on small bowel adaptation following massive bowel resection in rabbits (Eyarefe et al., 2008) revealed that honey had a good trophic effect on the residual bowel comparable to glutamine following resection. In this paper, we report our observation on the effect of honey, glutamine and their combination on bowel wall and mucosa hyperplasia following 70% small bowel resection in some local dogs in Nigeria.

MATERIALS AND METHODS

Experimental Animals

Twenty four (24) non-descript local dogs, age 3-5 months, of either sexes with mean body weight of 4.42 ± 0.70 kg were studied. Ethical clearance was obtained from the ethical committee of the Faculty of Veterinary Medicine, University of Ibadan, before the commencement of the experiment. The dogs were sourced from local breeders in Ibadan, Nigeria, housed in the experimental animal unit, Department of Surgery and Reproduction, University of Ibadan, in individual cages that provided ample space for exercise and they were fed with balanced, compounded diet, and water ad libitum. The dogs were conditioned for three weeks and judged to be of good general health based on complete clinical examinations before the commencement of the experiment. All experimental protocols and handling were in compliance with the NIH publication No 85-23 guidelines (NIH publication revised, 1985)

Anaesthesia

Each animal was deprived of food 8 hours prior to surgery but had access to water ad-libitum. They were premedicated with 1ml injection of 3% Pentazocine (Fortwin-Ranbaxy pharmaceuticals Ltd, India) at the dose rate of 5mg/kg and 2% Xylazine (Kepro, Holland) at the dose rate of 0.5mg/kg. Surgical anaesthesia was achieved with 2.5% Thiopentone sodium at a dose of 10mg/kg through pre-placed scalp vein set via cephalic venupuncture.

Experimental design and surgical Procedure

The ventral abdomen was aseptically prepared, and the intestine approached through a ventral midline abdominal incision. The intestinal loops were exteriorized over saline moistened laparotomy sponges. The Treitz ligament was located and the small bowel length was determined as earlier described (Eyarefe et al, 2008) from the Treitz ligament to the ileocolic junction. Seventy percent (70%) of the small bowel length from 10cm distal to the Treitz ligament was resected. The residual bowel segment was anastomosed with Polyglactin 910 (Vicryl ® Ethicon, USA), in an end to end anastomosis as earlier described (Orsher et al, 1993). The laparotomy incision was closed using a standard surgical technique. A 3cm full thickness biopsy section was obtained from the excised segment and fixed in 10% formalin (Pre-treatment sample). Animals were placed in recovery cages, and maintained on intravenous dextrose solution until animals could drink and eat 24-48 hour post resection before being returned to the experimental animal cages. Following resection, the dogs were randomised into four treatment groups (A, B, C and D). Group A dogs (n=6) were placed on oral glutamine (33g/5kg per day), Group B dogs (n-6) on oral honey (10 ml) and glutamine (33g/ 5kg per day), Group C dogs (n-6) on oral honey (10ml), and Group D dogs water (control group). All groups were supplied with adequate food and water. 3cm full thickness post-treatment biopsy samples were obtained from two representatives of each group and fixed in 10% formalin following a 2nd surgery at days:7, 14 and 28, and the residual small bowel segment evaluated for healing, and gross changes in length . The formalinized tissues were routinely processed for histomorphometric studies.

Body Weight evaluation
Animal body weights changes were evaluated at three days intervals for fifteen days to evaluate the influence/effects of the various treatments on body weight using a weighing scale.

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, (2005). For each parameter, 10 villi and crypts were evaluated and an average values calculated.

Statistical Analysis
All data are expressed as means ±SD. Differences among groups were evaluated by a one way analysis of variance (ANOVA) followed by a two-tailed Student's t-test. P-values less than 0.05 were considered to be statistically significant.

RESULTS

Effects of treatment on body weight
Animals in all the groups experienced an initial fall in body weight from days 0-3 post surgery. Animal on glutamine/honey combination, glutamine and honey had gradual increase in body weights from days 3-15 of weight evaluation. The control group, however, had a remarkable drop in body weight compared with other groups. (Fig 1)

Effects of treatment on villi height
Animals in all the treatment groups experienced some increase in mucosal villi height. Those of glutamine, glutamine/honey and honey were significant at $P<0.05$ when pre-treatment and post treatment values were compared (fig 2).

Effects of treatment on villi width
Animals in all the treatment groups experienced some increase in mucosal villi width. Those on, Glutamine/honey, glutamine and honey were significant at $P<0.05$. (Glutamine/honey> glutamine> honey) (Fig 3).

Effects of treatment on cryptal depth
Animals in all the treatment groups experienced some increase in mucosal cryptal depth. The increase in animals on glutamine was significant at $P<0.05$, while those on honey and glutamine /honey combination were remarkable but not significant at $P< 0.05$ (Fig 4).

Effects of treatment on villi density
A comparison of the villi densities showed that glutamine /honey combination had the best influence/effects overall compared with animals on glutamine, honey and control (Fig 5).

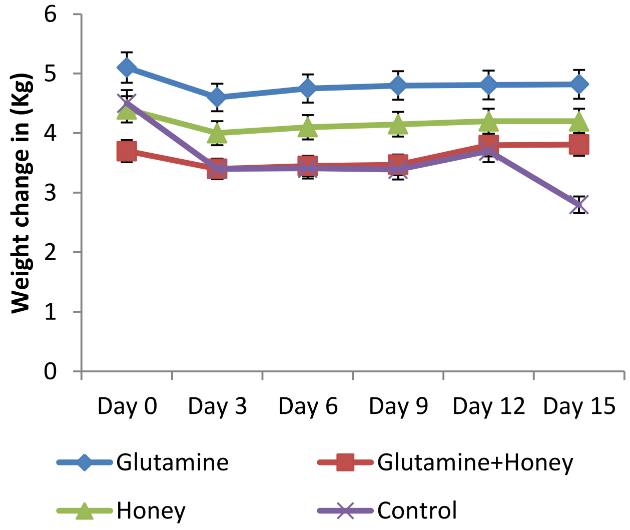

Figure **1:** Changes in body weight in glutamine, glutamine-honey, honey, and control groups

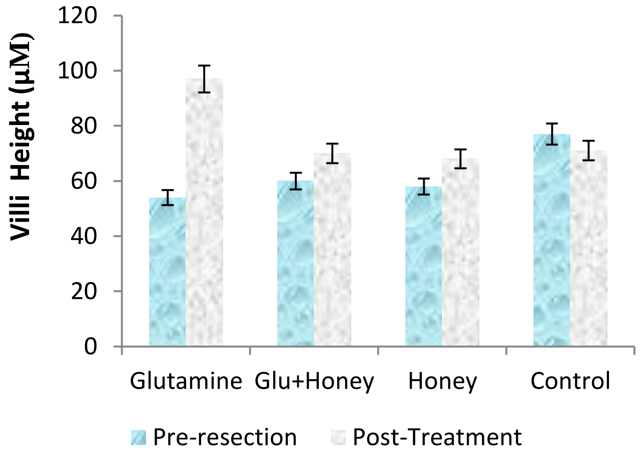

Figure 2: Comparison of pre-resection and post-treatment villi height of dogs bowels treated with glutamine, glutamine and honey, honey and the control groups

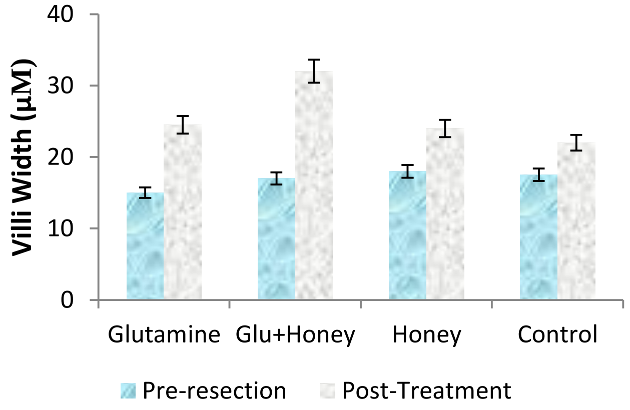

Figure 3 **:** Comparison of Pre-resection and Post-treatment Villi Width of Bowels of Dogs treated with Glutamine, Glutamine and Honey, Honey and the Control groups

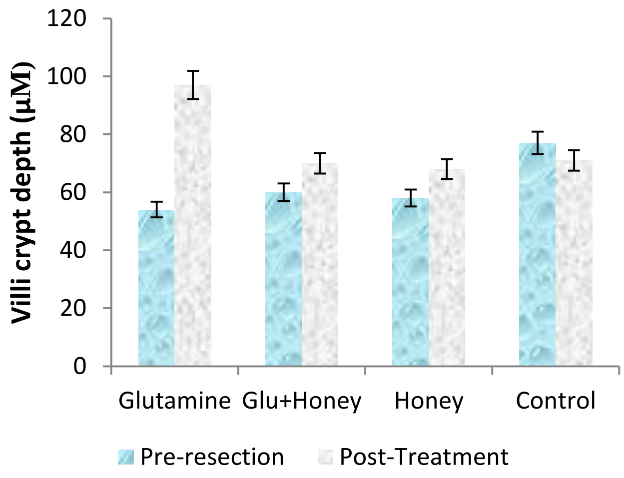

Figure 4: Comparison of pre-resection and post-treatment crypt depth of bowel of dogs treated with glutamine, glutamine and honey, honey and the control groups.

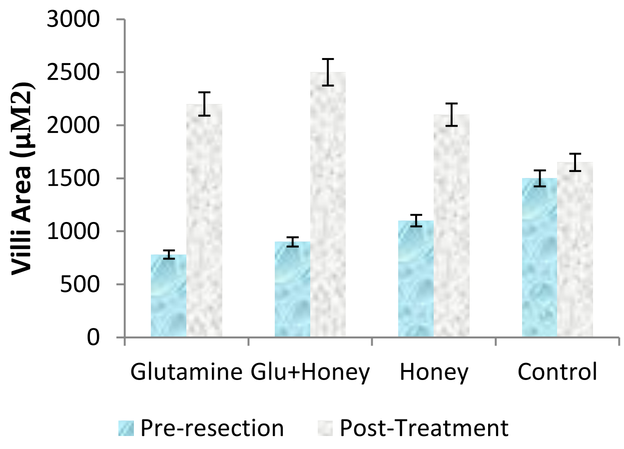

Figure 5**:** Comparison between pre- and post-resection villi cross sectional area of bowel of dogs treated with glutamine, glutamine and honey , honey and the control.

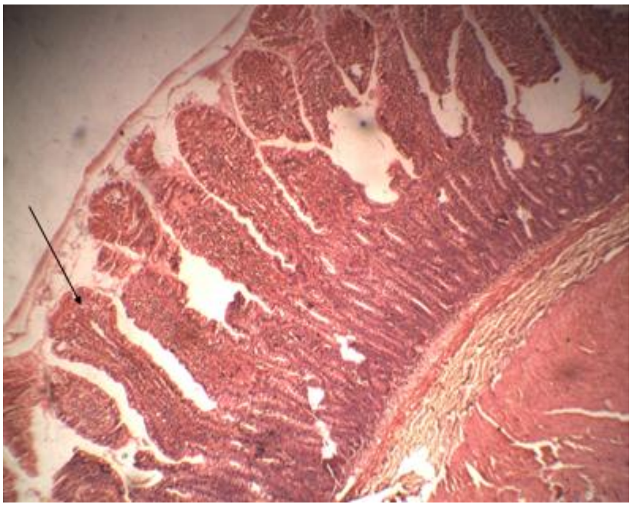

Figure 6: The photomicrograph of the intestine of dogs treated with glutamine –honey combination following 70% small bowel resection with unique 'fan shaped' Villi Branching. (Mag x 150 H&E)

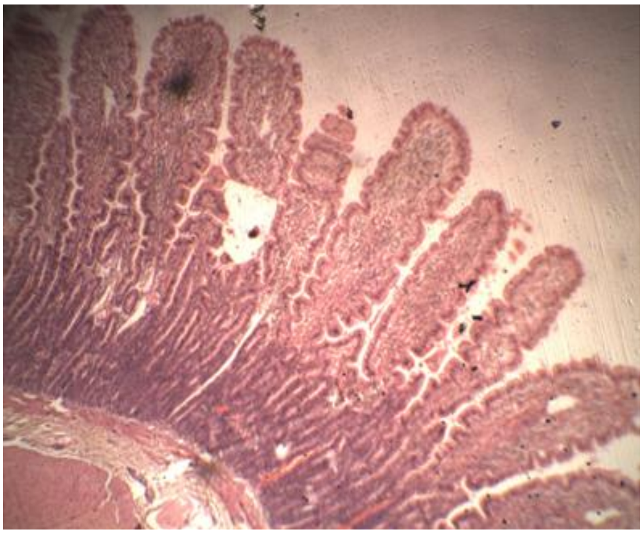

Figure 7: The photomicrograph of the intestine of dogs treated with honey following 70% small bowel resection with serrated villi. (Mag x 150 H&E).

DISCUSSION

The result of this study shows that glutamine/honey, glutamine and honey have beneficial effects on intestinal adaptation processes following massive resection. It also shows that glutamine/honey combination have a comparative therapeutic advantage over either glutamine or honey. Eyarefe et al, (2008) had earlier reported the effects of honey and glutamine on the gut adaptive process following 50 % small gut resection in rabbits. This report further reaffirms the effects of honey and glutamine on the gut adaptive process, and the observed effects of the combination therapy which is the first to be reported in literature, showed the synergistic trophic effect.

The passing of diarrheic faeces among the groups between 1st to 3rd post operative days was consistent with findings in similar studies (Rombeau *et al.*, 1987, Eyarefe *et al.*, 2001, Eyarefe *et al.*, 2008). Adaptive changes have been reported to begin 12-24 hours after massive intestinal resection and will continue for more than a year after resection along with bowel compensation, and reduction in diarrhoea and malabsorption (Rombeau et al, 1987, AGA, 2003). The initial weight loss observed in this study was consistent with those observed by other investigators (Joaquim et al, 2005) and this has been associated with surgical stress and tissue catabolism associated with wound healing (Eyarefe et al, 2001). It could also be attributed to the post resection diarrhea, and the reduced capability of the residual bowel to digest and absorb nutrients at the early post operative period (Lentze, 1989). The observed diarrhea subsided in all the groups from the third postoperative day**.** It correlated with the gradual increase in body weight gain observed in this study (fig 1). The increase in weight could be associated with the increase absorptive surface area due to the increase in villi height, width and cryptal depth as earlier reported in rabbits (Eyarefe et al., 2008). The synergy expressed in the glutamine-honey treatment buttressed by the fan-shaped villi with serrated edges (fig. 6, 7), showed that honey contain trophic substance which in combination with glutamine enhanced regeneration of intestinal epithelium. Honey has been widely reported to have antibacterial properties (Effem et al,1992, Molan,1992, Molan,1997) antioxidant, immune boosting **(**Molan,2001**)**, and tissue regenerating effects (Bansal 1985). Glutamine is also known to enhance gut mucosal growth, repair and function, decreases gut associated sepsis and improves nitrogen balance in animal models of intestinal atrophy, injury and adaptation as well as maintenance of Gut Associated Lymphoid Tissues (Jacobs et al, 1988, O'Dwyer et al, 1989, Lian-An and Jie-Show, 2003 Satoh et al., 2003). The combined effects of the duo may have been responsible for the observed improved adaptation of this group.

This study in dogs has also shown that the application of honey and glutamine could be of immense therapeutic benefits to other animals and human subjects with SBS, since the dogs' gastrointestinal tract is similar to that of man (Ellenport,1975). Furthermore, the observed synergy produced by the honey-glutamine therapy is of immense importance as this showed the effect of combining synthetic drugs and natural product in the management of massive intestinal resections. This study also reveals that in resource poor setting where glutamine is not affordable, similar effects can be achieved with honey thereby cutting down the cost of caring for these patients (Fernando et al., 2009). Based on the results of this study we therefore postulate that honey/glutamine combination have comparative therapeutic advantage over glutamine or honey and may be a preferred treatment option for short bowel syndrome patients.

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