Indian Journal of Experimental Biology
Vol. 51, April 2013, pp. 303-312

Comparative effect of cane syrup and natural honey on abdominal viscera of growing male and female rats[†]

Abdulwahid Ajibola[1,3*], Joseph P Chamunorwa[2] & Kennedy H Erlwanger[3]

[1]Department of Veterinary Physiology & Pharmacology, Faculty of Veterinary Medicine, University of Ilorin, P.M.B 1515, Ilorin, Kwara State, Nigeria
[2]Department of Veterinary Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa
[3]School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa

Received 10 September 2012; revised 1 January 2013

The high intake of refined sugars, mainly fructose has been implicated in the epidemiology of metabolic diseases in adults and children. With an aim to determine whether honey can substitute refined sugars without adverse effect, the long-term effects of natural honey and cane syrup have been compared on visceral morphology in growing rats fed from neonatal age. Honey increased the caecum and pancreas weights in male rats, which could enhance enzymatic activities of pancreas and digestive functions by intestinal microflora of caecum. Unlike honey, cane syrup caused fatty degenerations in the liver of both male and female rats. Honey enhanced intestinal villi growth, and did not cause pathology in the rodents' abdominal viscera, suggesting potential nutritional benefit as substitution for refined sugars in animal feed.

Keywords: Abdominal viscera, Cane syrup, Gastrointestinal tract, Growing rats, Honey

Consumption of fructose-rich diets alters gastro-intestinal functions[1] with consequent morphological and physiological changes in the visceral organs[2]. The lifestyle of modern day persons, especially children involve a high intake of junk and fast foods with added sugars, mainly sucrose and high fructose corn syrup[3]. The culpability of these two sources of dietary fructose in the epidemiology of kidney, cardiovascular and metabolic diseases is well established[1,4]. Natural honey (NH) is a sweet and flavoured natural product with high nutritive value[5], while cane syrup is a thick, amber-coloured artificially refined sucrose-rich syrup derived from sugar cane. Both honey and cane syrup are liquid foods containing mainly fructose as sugars. Their dietary effects have been extensively investigated in adult animal models[6-9]. However, there is a dearth of literature on the long term effects of these two substances in neonates. There is anecdotal report encouraging the feeding of honey to newborn babies, supported by cultural and traditional practices[9]. The inclusion of refined sugars in infant formula has also been documented[10]. The gastrointestinal tract (GIT) is the first contact point for food and other orally administered substances. When food materials are ingested, the structural integrity of the GIT can be affected, and consequently influence the GIT's functional aspects. The structure of the GIT can also change in response to different dietary constituents, which can enhance functional or physiological changes in intestinal and visceral organs such as cellular growth and functions of intestinal transporters[11], with consequent increase in size[12]. The GIT is a potent source of regulatory peptides[13], such as cholecystokinin, secretin, gastrin, ghrelin and motilin[14]. The presence of food in the duodenum stimulates production of the intestinal hormone, cholecystokinin which stimulates exocrine pancreatic activity, leading to the secretion of an array of hydrolytic enzymes. Dietary manipulations during the peri-natal suckling period have long lasting and apparently irreversible effects on some transport mechanisms in the GIT[11]. The few available data on

*Correspondent author
Telephone: +234 8037803988, +234 37781041
Fax: +234 31222561
E-mail: ajibola66@gmail.com

[†]Part of this work was accepted for oral presentation as an abstract titled "Comparative effects of honey and sugar on the morphometry of viscera in growing Sprague-Dawley rats (*Rattus norvegicus*)" at the 31st Scientific Conference, Physiological Society of Nigeria (PSN) held at Usmanu Dan Fodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria during 7–9 September, 2011.

honey-fed young animals commence with weaned animal models[7,15], and do not take cognisance of possible gender differences. This study compared the long term effects of two main sources of dietary fructose [natural honey (NH) and golden syrup] on the abdominal visceral organs of growing male and female Sprague-Dawley rats.

Materials and Methods

Animals—Neonatal (7-day old male and female) Sprague-Dawley rats (59) with a mean body weight of 17.9±0.28), (range 14–22) g from six dams supplied by Central Animal Services, University of the Witwatersrand, Johannesburg, South Africa were used for the study. The pups with their respective dams were housed as a family in solid bottom plastic cages (425 × 270 × 140 mm) with beddings of wood shavings. They were placed on a 12 : 12 h light-dark cycle (lights on 0700–1900 hrs) and environmental temperature of 22±2 °C. The dams were given commercial rat feed (CRF) (Epol®, Johannesburg, South Africa) and tap water *ad libitum.*

Dietary supplements—The supplements used for the dietary treatments were cane syrup in the form of golden syrup (GS golden syrup®, GS (Illovo Sugar Ltd, Illovo, Natal 4150, South Africa); natural honey (monofloral Sunflower honey®, Willy's B Farm, Eikenhoff 1872, South Africa) and distilled water.

Study design—The study was approved by the Animal Ethics Screening Committee (AESC approval number–2010/29/2B) of the University of the Witwatersrand, South Africa. It was also performed according to the humane handling rules contained in the "Guidelines for the use and care of animals in Experimental, Education and other Scientific Procedures" of the University of the Witwatersrand, South Africa. The study involved dietary treatments with natural honey or cane syrup which were given to the rats up to 13 weeks of age. The pups in each litter were randomly divided into five male groups, with replication of five female groups as well.

Dietary treatments—The pups were administered with 0.1 mL of either distilled water, or GS or NH according to their respective dietary treatments for the first three days of the study (age 7–9 days), in order to familiarise them with the experimental procedures of handling and gavaging. The pups were later administered with either a low or high dose of the treatments (NH or GS) once a day, from day 10 to 21 postpartum as per their groups.

The groups were: Group 1: control, distilled water; Group 2: golden syrup low (GSL); Group 3: natural honey (NHL); Group 4: golden syrup high (GSH); Group 5: natural honey high (NHH). The low doses were given at 10 mL kg^{-1} of 50% solution, whilst high doses were given at 20 mL kg^{-1} of 50% solution once a day by oral gavaging using a plastic orogastric gavaging needle and 1mL hypodermic syringe. The dilution of the golden syrup and honey was done so as to avoid gavaging the young rats with thick substances that could clog their respiratory tracts. The pups were kept with their respective dams to suckle between gavages and weighed daily before weaning to monitor growth. There were six rats in each group except NHH females group that had five animals, due to unequal number of littermates produced by the dams. In the undiluted form, the substances used were iso-caloric and each had less than 0.5% protein content (Table 1). The proximate analyses shown in Table 1 were as determined by the South African National Accreditation Systems (SANAS) accredited Analytical laboratory (Irene Analytical Services, Agricultural Research Council, South Africa).

The rats were weaned at 22 days of age, and the dams were returned to stock. The weaned rats were then housed in pairs in the solid bottom plastic cages (425 × 270 × 140 mm) provided by Central Animal Services. The experimental animals were placed on a 12 h L: D cycle (lights on 0700–1900 hrs) and environmental temperature of 22±2 °C. The solutions were then added to the commercial rat feed (CRF) at the rate of 20% of the diet (v/w) for the low dose groups, while high dose groups had 50% solutions mixed with their respective diets (v/w). The CRF group had tap water added to its diet post-weaning as 20% of the diet (v/w). All the experimental rats were supplied with tap water *ad libitum* for drinking.

The diets were prepared daily, weighed and freshly served in clean ceramic bowls to the animals according

Table 1—Nutritional composition (dry matter basis) of the undiluted forms of natural honey (NH) and golden syrup (GS)

[Values are mean±SE].

Proximate analyses	Natural honey (NH)	Golden syrup (GS)
Dry matter (%)	84.08±0.07	83.38±0.01
Energy(MJ/kg)	15.56±0.21	15.55±0.06
Protein* (%)	0.42±0.06	0.25±0.05
Fat (%)	0.53±0.01	0.62±0.00
Ash (%)	0.53±0.00	0.17±0.00

*Obtained by multiplying nitrogen content by the factor of 6.25.

to their treatment groups. The nutritional composition of the adult diets as determined by the SANAS accredited Analytical laboratory (Irene Analytical Services, Agricultural Research Council, South Africa) is shown in Table 2. All the diets used were iso-caloric and dose-matched diets were iso-nitrogenous (Table 2). The control, CRF diet was also iso-nitrogenous with low dose-diets. The doses of all the dietary treatments were within the ranges of previous studies in rats[6-8,15,16]. After weaning, each animal was weighed twice weekly to monitor body weight.

Visceral organs' measurements—At the age of 13 weeks, the rats were euthanized by intra-peritoneal injection of sodium pentobarbitone (150 mg kg^{-1}) (Euthanaze®, Centaur Laboratories, Johannesburg, South Africa), and meticulously dissected for gross and microscopic morphometric measurements. The lengths of the small and large intestines were recorded by dissecting out the intestines and arranging each on a straight line with minimum stretching on a board[17]. The weight of the abdominal visceral organs was obtained with Precisa 310M digital balance (Precisa®, Vadodara, Switzerland).

Portions of the small intestine (from the middle segment), 1 cm long were fixed in 10% formal saline, embedded in paraffin blocks, then cut into 8 µm thick sections, and later stained with haematoxylin and eosin (H & E) for histological examinations. The jejunum was used for histology due to its absorptive importance. Morphometric measurements were done with an eye piece micrometer mounted on a light microscope (LM) (Reichert®, Austria) at 100x magnification. The mean villous height per segment was estimated as the mean of the measurements (of 3–5 sections per rat)[18]. In an attempt to minimise the error of under-estimation due to angle and position of section planes, only the tallest profiles in each segment were measured[19].

Portions of the caudate lobe of the liver were cut from each liver sample. The caudate lobe of the liver was used for consistency of observation because the histology of the liver is distinct from that of other organs in that every 3–4 cells (lobules) of it have their own vessels and their own drainage systems. These liver sections were also fixed in 10% formal-saline and later stained with H & E stain to prepare histological sections of 5µm thickness. The sections were examined and assessed under LM for cellular damage and fatty degeneration.

Statistical analysis—Data were expressed as mean ± SE. GraphPad Prism for Windows Version 5.02 (GraphPad Software, San Diego, California, USA) was used for data analyses. Student's *t*-test was used for the analysis of NH and GS shown in Table 1. One way analysis of variance (ANOVA) with Neuman-Keuls post hoc was used as a multiple comparison test. A $P < 0.05$ was considered significant for any observation, unless otherwise stated.

Results

Body weight (BW)—The growth pattern records showed that the rats in all groups had significant body weight gain (BWG) from the commencement to the end of the study at 13 weeks of age (Table 3). However, the final BW and BWG of the NHL male group was significantly higher ($P< 0.01$) than that of the other male treatment groups, and normal slightly higher (5.0%) relative to the control, CRF rats (Table 3). There was no significant difference ($P > 0.05$) in the final BW among the female groups, and consequently the difference in BWG did not attain any significance ($P > 0.05$) among the females.

Gross measurements—The absolute weights (g) and lengths (cm) of the small intestine (SI) and large intestine (LI) showed significant differences ($P < 0.05$) amongst the male as well as the female

Table 2—Nutritional composition (dry matter basis) of control and treatment diets used during the post-weaning period

[Values are mean±SE]

Proximate analyses	CRF	GSL	NHL	GSH	NHH
Dry matter (%)	74.79±0.09^a	83.18±0.22^b	83.49±0.21^b	76.75±0.48^a	76.61±0.02^a
Energy(MJ/kg)	16.47±0.04^a	17.03±0.24^a	16.56±0.06^a	17.11±0.19^a	17.87±0.01^b
Protein* (%)	19.33±1.49^a	17.00±0.08^a	17.50±0.46^a	11.43±0.57^b	11.85±0.30^b
Fat (%)	3.71±0.01^a	4.43±0.03^b	3.51±0.02^c	2.08±0.02^d	2.45±0.01^e
Ash (%)	7.74±0.04^a	5.95±0.06^b	6.30±0.07^c	3.72±0.08^d	4.42±0.02^e

*Obtained by multiplying nitrogen content by the factor of 6.25; Data in the same row with different superscripts are statistically significant ($P < 0.05$); CRF = commercial rat feed; GSL = golden syrup low; NHL = natural honey low; GSH = golden syrup high; NHH = natural honey high.

groups (Table 4). However, there were no significant differences ($P \geq 0.05$) in the relative lengths per cent body weight and weight : length ratio (g cm^{-1}) of these organs (SI and LI) in both sexes (Table 4). There were gender differences shown in these parameters with the females having lower values relative to corresponding male values.

There were significant differences ($P < 0.05$) in the absolute (g) and relative (%) weights of the male abdominal visceral organs namely caecum, pancreas and visceral fat, while there were no significant differences in gross measurements of same organs amongst the female groups (Table 5). The absolute kidneys' weight of the male rats at high dose of both diets (GSH and NHH) were reduced ($P < 0.05$), however the relative measurements (%) showed that there was also no difference ($P \geq 0.05$) amongst all the groups (Table 5).

Histological assessment of SI and liver sections—Microscopic examinations of the small intestinal villi and crypts showed reduced height and width of the villi, and crypt depth, as well as the SI villus height : crypt depth ratio in the male rats fed cane syrup supplements and control rats, relative to the NHL group (Fig. 1). In the female groups, both diets at low dose (GSL and NHL) increased ($P < 0.05$) the SI villus height and crypt depth than those of the high dose-diets (GSH and NHH) and control rats (Fig. 2). The honey fed female rats had normal SI villus width relative to control (CRF) rats, and higher ($P < 0.05$) compared to the GS-fed female rats; whilst the SI villus height : crypt depth ratio did not differ ($P > 0.05$) amongst the female rats.

The hepatic changes observed under the LM were also recorded in Figs 3 and 4. The rats fed CRF and the two doses of NH had liver sections showing normal

Table 3—Initial and final body weights (BW), and body weight gain (BWG) of the experimental rats

[Values are mean±SE from animals in each group].

Body weight (g)	Sex	CRF	GSL	NHL	GSH	NHH
Initial weight (g)	M	17.2±0.31	16.8±0.83	18.0±0.68	18.0±0.83	19.7±0.67
	F	17.3±1.05	17.3±0.42	18.0±0.45	17.5±0.56	18.6±0.51
Final weight (g)	M	492.2±8.92*	443.0±9.04	516.7±11.51*	435.7±6.61	426.0±9.75
	F	276.8±3.17	271.5±5.66	295.8±8.15	271.5±13.08	282.0±10.60
Weight gain (g)	M	475.0±8.76*	426.2±8.34	498.7±11.35*	416.8±5.83	406.3±9.44
	F	259.5±2.58	254.2±5.94	277.8±7.89	257.0±13.6	263.4±10.32

*significantly different ($P < 0.01$) to others along the row.

Table 4—Gross measurements of small intestine and large intestine of the experimental rats

[Values are mean±SE].

Intestine		Sex	CRF	GSL	NHL	GSH	NHH
Small intestine	Weight (g)	M	$8.09\pm0.30^{a,c}$	9.41 ± 0.57^{b}	$9.05\pm0.20^{b,c}$	$8.26\pm0.13^{a,c}$	7.84 ± 0.30^{a}
		F	6.42±0.16*	6.58±0.24*	6.70±0.29*	6.50±0.33*	6.19±0.14*
	Length (cm)	M	$131.75\pm0.98^{a,c}$	140.75 ± 4.00^{b}	$138.33\pm0.67^{b,c}$	128.00 ± 1.37^{a}	$130.50\pm2.43^{a,c}$
		F	123.00±2.22*	130.40±3.84*	124.00±2.06*	124.60±2.90*	113.40±4.86*
	Weight: Length ratio (g cm^{-1})	M	0.06±0.00	0.07±0.00	0.07±0.00	0.07±0.00	0.06±0.00
		F	0.05±0.00	0.05±0.00*	0.05±0.00*	0.05±0.00*	0.06±0.00
	Weight (%)	M	1.86±0.04	2.01±0.05	1.96±0.09	1.96±0.05	1.92±0.05
		F	2.45±0.09*	2.27±0.17	2.41±0.07*	2.38±0.06*	2.27±0.08
Large intestine	Weight (g)	M	$2.29\pm0.11^{a,c}$	$2.41\pm0.11^{b,c}$	$2.18\pm0.11^{a,c}$	1.98 ± 0.07^{a}	2.03 ± 0.06^{a}
		F	1.60±0.10	1.73±0.08	1.80±0.09	1.82±0.11	1.67±0.05
	Length (cm)	M	24.00±0.33	23.92±1.03	23.33±0.69	24.33±0.71	24.33±0.42
		F	20.70±0.73	20.60±0.55	20.20±0.53	20.90±0.77	21.60±0.86
	Weight: Length ratio (g cm^{-1})	M	0.10±0.01	0.10±0.00	0.09±0.00	0.09±0.00	0.08±0.00
		F	0.08±0.00	0.08±0.00	0.09±0.01	0.09±0.00	0.08±0.00
	Weight (%)	M	0.53±0.02	0.51±0.02	0.47±0.03	0.47±0.02	0.50±0.01
		F	0.61±0.04	0.60±0.05	0.65±0.02	0.67±0.03	0.61±0.03

Data in the same row with different superscripts are significantly different ($P < 0.05$). *values different at $p < 0.05$ from the corresponding values of male group.

Table 5—Absolute (g) and relative (% BW) weights of the abdominal visceral organs of the experimental rats [Values are mean±SE].

Organ	Unit	Sex	CRF	GSL	NHL	GSH	NHH
Liver	g	M	11.16±0.34	14.23±0.56*	10.92±0.32	12.57±0.30*	10.24±0.40
		F	7.02±0.33$^{\beta}$	7.44±0.34$^{\beta}$	7.49±0.30$^{\beta}$	7.74±0.41$^{\beta}$	7.20±0.28$^{\beta}$
	%	M	2.57±0.04	3.03±0.15*	2.72±0.13	2.57±0.04	2.51±0.06
		F	2.67±0.10	2.55±0.18$^{\beta}$	2.70±0.06	2.83±0.05	2.63±0.04
Caecum	g	M	1.62±0.06	1.45±0.07	1.73±0.05	1.38±0.06	1.49±0.09
		F	1.23±0.08	1.15±0.06	1.19±0.04$^{\beta}$	1.18±0.10	1.05±0.04
	%	M	0.37±0.01*	0.31±0.01	0.38±0.02*	0.33±0.01	0.37±0.02*
		F	0.46±0.02	0.40±0.04	0.43±0.02	0.43±0.03	0.39±0.02
Stomach	g	M	2.31±0.10	2.12±0.08	2.46±0.13	2.23±0.09	2.33±0.06
		F	1.85±0.07	1.72±0.04	1.77±0.05	1.76±0.10	1.61±0.12
	%	M	0.54±0.03	0.46±0.02*	0.55±0.02	0.52±0.01	0.55±0.02
		F	0.71±0.03	0.60±0.05	0.64±0.02	0.64±0.02	0.60±0.06
Pancreas	g	M	1.80±0.10	2.20±0.16*	2.52±0.11*	1.73±0.12	2.25±0.09*
		F	1.41±0.08	1.66±0.07	1.68±0.13	1.51±0.22	1.41±0.10$^{\beta}$
	%	M	0.42±0.03	0.47±0.04	0.55±0.03*	0.41±0.03	0.56±0.04*
		F	0.54±0.03	0.57±0.05	0.61±0.04	0.54±0.06	0.52±0.04$^{\beta}$
Spleen	g	M	1.16±0.04	1.23±0.06	1.25±0.06	0.94±0.04*	0.96±0.04*
		F	0.93±0.03	0.87±0.01	0.91±0.02	0.88±0.07	0.78±0.02
	%	M	0.27±0.01	0.26±0.01	0.27±0.02	0.22±0.01*	0.23±0.01
		F	0.36±0.02	0.30±0.02	0.33±0.01	0.32±0.02$^{\beta}$	0.29±0.01
Kidneys	g	M	2.99±0.07	3.21±0.11	3.11±0.13	2.56±0.08*	2.53±0.07*
		F	1.96±0.07$^{\beta}$	1.91±0.05$^{\beta}$	1.95±0.08$^{\beta}$	1.95±0.10$^{\beta}$	1.82±0.05$^{\beta}$
	%	M	0.69±0.01	0.68±0.02	0.68±0.05	0.60±0.01	0.62±0.01
		F	0.75±0.03	0.66±0.05	0.70±0.00	0.72±0.03	0.67±0.03
Visceral fat	g	M	8.56±0.50	17.70±0.73*	11.11±0.37	15.48±0.85*	10.21±1.04
		F	6.68±0.66*	10.25±1.00$^{\beta}$	8.17±0.66	10.09±1.32$^{\beta}$	9.20±0.92
	%	M	1.97±0.13	3.75±0.14*	2.39±0.07	3.64±0.14*	2.48±0.21
		F	2.56±0.28	3.48±0.34	2.96±0.26	3.65±0.36	3.39±0.38

*Data in the same row with superscripts are significantly different ($P < 0.05$). $^{\beta}$value significantly different at $P < 0.05$ from the corresponding value of male group.

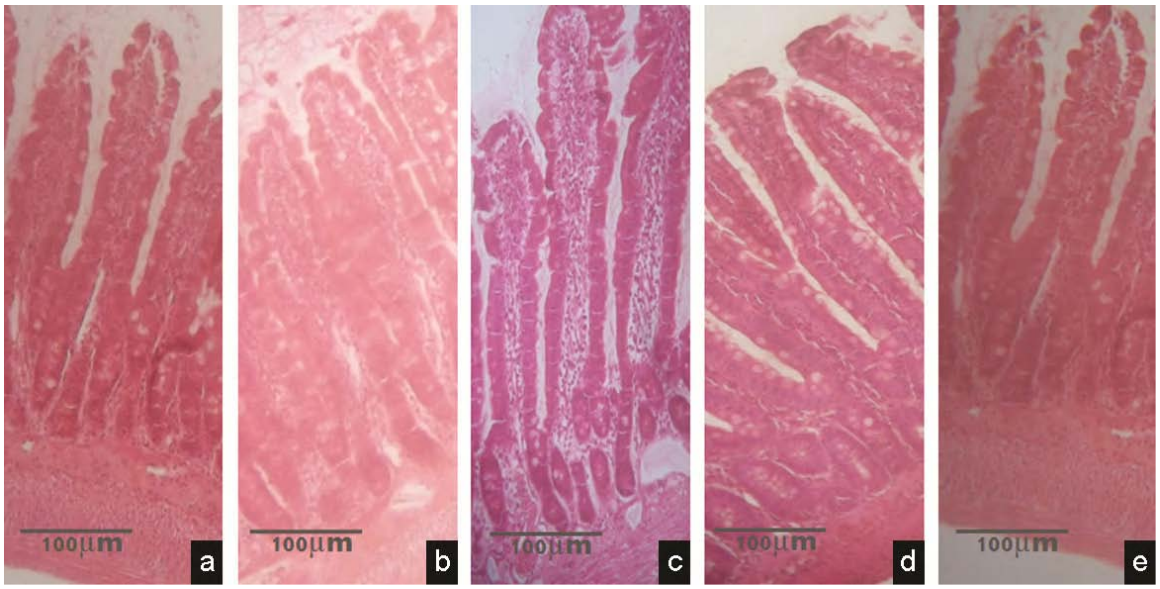

Fig. 1—Photomicrographs of male small intestinal villi [(a) CRF, control group; (b) GSL; (c) NHL; (d) GSH; (e) NHH; GSL and NHL villi height significantly higher than the villi height of GSH, NHH and control rats; NHL villus height significantly higher than the GSL villus height but not different from the control (CRF) villus height; SI villus height : crypt depth ratio in the NHL is significantly higher than that of GSL and control rats. (Haematoxylin & Eosin stain, magnification 100 x), Scale bar-100µm]

cytology. There were various degrees of fatty degenerations in hepatic sections of GS-fed animals (Figs 3 and 4).

Discussion

The growth pattern of all the experimental animals showed that the treatment diets resulted in significant weight gain at the end of the study. However, in the male rats, NH resulted in a significantly higher BWG than the other rats. There was no difference in BWG of the female groups throughout the study. Although, honey increased the absolute and relative weights of some visceral organs which included caecum,

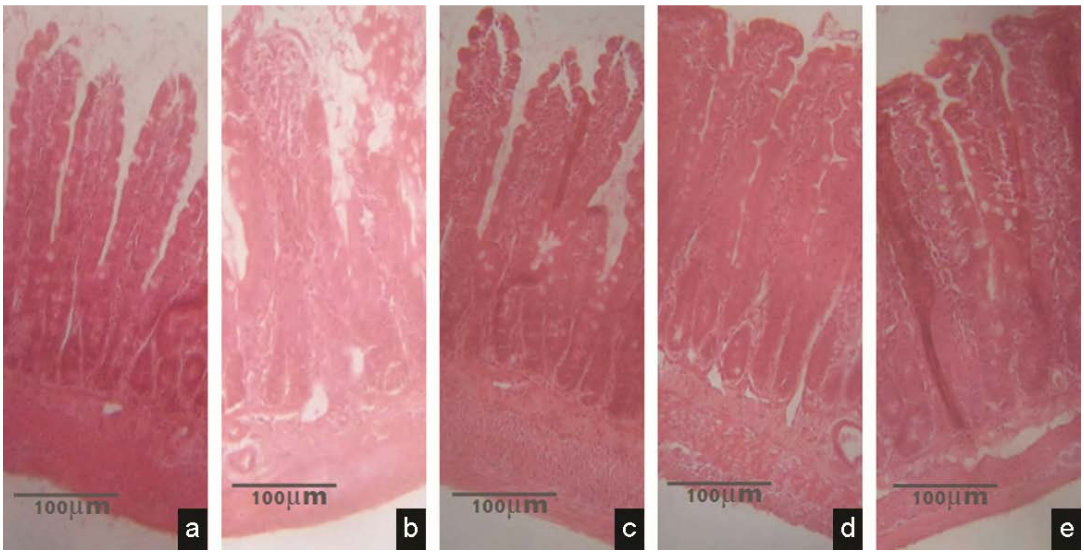

Fig. 2—Photomicrographs of female rat small intestinal villi [(a) CRF, control group; (b) GSL; (c) NHL; (d) GSH; (e) NHH; GSL and NHL villi height significantly higher ($p < 0.05$) than the villi height of GSH, NHH and control rats; NHL villus height significantly higher ($p < 0.05$) than the GSL villus height but not different from the control (CRF) villus height. (Haematoxylin & Eosin stain, magnification 100 x), Scale bar-100µm]

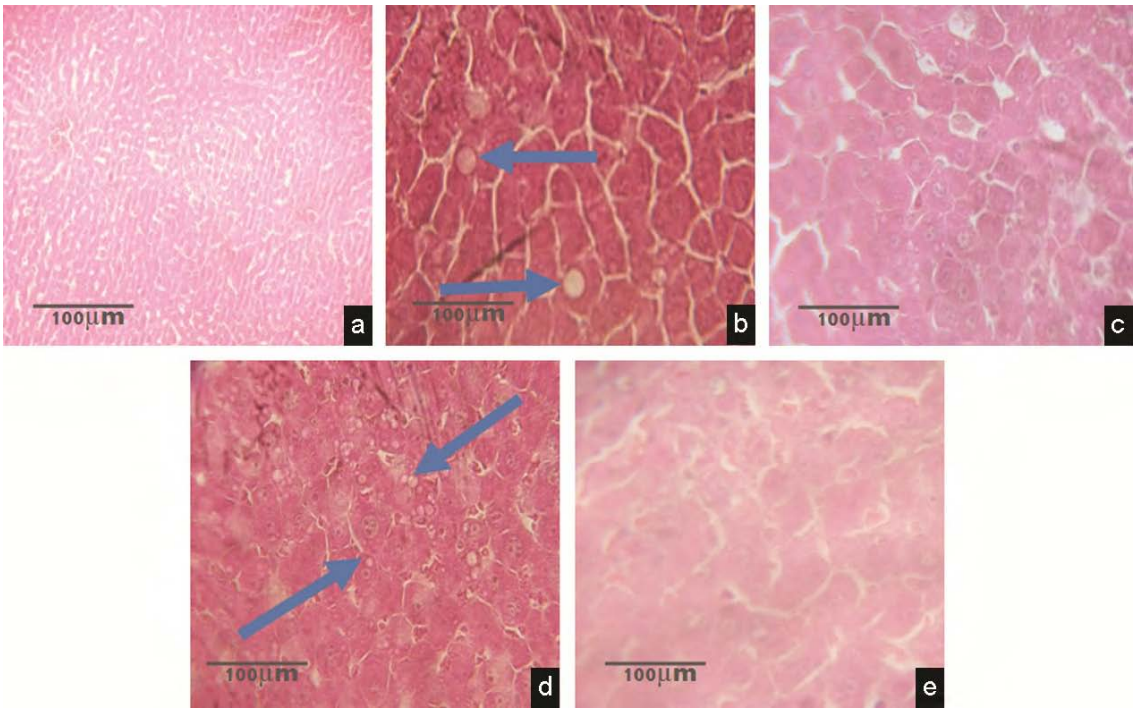

Fig. 3—Photomicrographs of male rat liver [(a) CRF, control group; (b) GSL; (c) NHL; (d) GSH; (e) NHH; magnification (mag) of control liver is 100 x, while treatment groups' livers mag is 400 x; Arrows showed fat droplets in GSL liver and fatty degeneration in GSH liver, while CRF, NHL and NHH showed normal cytology. (Haematoxylin & Eosin stain), Scale bar-100µm]

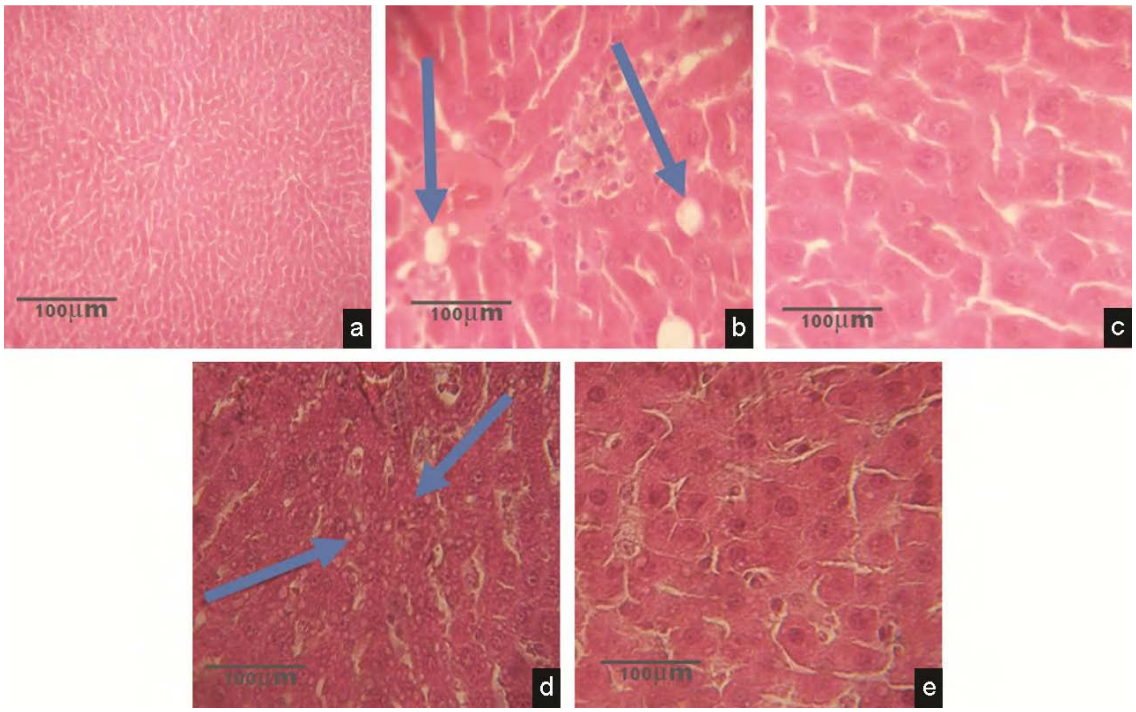

Fig. 4—Photomicrographs of female rat liver [(a) CRF, control group; (b) GSL; (c) NHL; (d) GSH; (e) NHH; magnification (mag) of control liver is 100 x, while treatment groups' livers mag is 400 x; Arrows showed fat droplets in GSL liver and fatty degeneration in GSH liver, while CRF, NHL and NHH showed normal cytology. (Haematoxylin & Eosin stain), Scale bar-100µm]

stomach, pancreas and spleen: the increase in BWG in the females was not significant. However, the organs' weight of honey-fed male rats was shown to have contributed positively to the animals' BWG.

The determination of organ weights is a long established practice to ascertain their functional integrity and establish health status of experimental animals[20]. There were differences in the absolute weights and lengths of the small and large intestines amongst all the animals. When these organs' weights were evaluated relative to the rats' body weights, the differences were not significant in both sexes. Further evaluation of the weight : length ratio (g cm^{-1}) of these organs also showed no significant difference amongst the groups. These plausibly suggested that the gross variations observed in weights and lengths were of no biological significance.

The predominant influence of natural honey on organs' macroscopic growth was shown by the caecum and pancreas in the male rats. The increased relative weight of the pancreas and caecum in the honey-fed male rats could enhance enzymatic production by the pancreas and digestive functions by intestinal microflora of the caecum. Previous studies with rats fed erythritol or alcohol extracts of African potato (*Hypoxis hemerocallidea*) resulted in significantly heavier caecal weight[21,22]. The mechanism underlying the increase in caecal weight is yet to be fully elucidated, but it was attributed to the trophic effect on the caecum by osmotically active or fermentable substances that are not absorbed from the small intestine[22]. Dietary manipulations can also affect microflora within the GIT. The previous analysis of honey's chemical constituents gives credence to the latter fact[23].

The absolute kidneys' weights of the male rats at high dose of both diets (GSH and NHH) were reduced ($P < 0.05$) than normal, however the relative measurements (%) showed that there were no significant differences ($P \geq 0.05$) amongst all the groups. Hence, the observed high organ weight of the low dose-diets (GSL & NHL) and control, CRF rats could not be of biological significance.

Contrary to honey's influence on healthy growth in the rodents, the GS-fed rats accumulated visceral fat, which accounted substantially for the BWG of these rats. The enhanced-visceral adiposity of the GS-fed rats was significant ($P < 0.001$) in males. This gave credence to the previous findings of reduced susceptibility of female rodents to sucrose induced metabolic adversity[6]. The GSL male group had a higher visceral fat weight ($P < 0.05$) than the high

dose-diet male rats, possibly due to volume of sucrose (GS) consumed. We estimated that low dose-diet groups ate more feed than their high dose-diet littermates (data not shown). The preferential intake of dry feed to pasty or liquid material (high dose-diets) by rats had been reported elsewhere[24].

The present study showed that none of the diets had significant biological effect on the gross morphology of the intestines when the weights were measured relative to BW. Thus, no experimental diet had any undue advantage over the other diets to influence the anatomical configuration and functionality of the GIT. The sections of the small intestines showed that NH had trophic effects on the intestinal cells of the male rats; whilst the SI villus height:crypt depth ratio showed that there was no difference ($P \geq 0.05$) amongst the female rats. However, the differential impact of the dietary treatments was observed at the microscopic level. In the male rats, the low honey group (NHL) showed enhanced development of the intestinal morphology than the other treatment and the control groups. The villous height, villous width and crypt depth of NHL rats were significantly higher ($P < 0.05$) than those of the other groups. Some workers reported an influence of high dietary fibre in their experimental rats where they found an increase in the number of intestinal cells[25]. Honey contains the biologically active soluble fibre, propolis[23], which could have produced the villi trophic effects. The influence of high dietary fibre on intestinal villi growth has also been documented elsewhere[26,27]. The NH-enhanced villi and crypts dimensions could have provided more surface area for digestive functions and enzymes activities in the GIT. According to some workers, the broader villi provide a greater surface area and, therefore, more brush border for nutrients absorption[19]. This could be the reason for improved growth of the honey fed rodents as emphasized in another study[26].

The comparison of the high dose-diets groups showed that all the GIT microscopic values did not differ significantly, except that the NHH villi had the same width as control, but wider ($P < 0.05$) than that of GSH rats. However, all the other intestinal morphological parameters obtained from NHH were higher, albeit insignificantly different than the corresponding values from high dose sucrose eaters (GSH). The enhanced intestinal villi growth of the honey eaters could improve their digestive functions with better feed utilisation. This agreed with the report from a study[19]on dietary supplementation that a decrease in the villus height to crypt depth ratio suggested reduced overall capacity for digestion and absorption of nutrients.

In the female animals, we recorded higher values ($P < 0.05$) of villus height and crypt depth for the groups in which the GS and NH were included at low doses (GSL & NHL) than those obtained from control and high dose groups (GSH and NHH), a reflection of higher estimated feed intake by the low dose groups, due to preferential dietary intake[24]. The villus width of honey-fed females was significantly larger than that of those animals nurtured with cane syrup (GS). Similar to our observation in male rats, all the microscopic measurements of high honey dose (NHH) female groups were higher than those obtained from their cane syrup (GSH) littermates. However, these values did not attain any statistical significance. The villus height to crypt depth ratio of our female rats did not differ.

The pathological trait associated with chronic cane syrup intake was further shown by the development of hepatomegaly in GS-fed animals. Contrary to GS induced hepatic abnormality, NH-fed rats had significantly attenuated absolute (g) and relative (%) liver weights compared to GS groups as shown in Table 5. The hepatic changes observed under the LM were also recorded in Figures 3 and 4. The rats fed CRF and those nurtured with either of the two NH diets had liver sections with normal cytology, while there were histopathological changes in the livers of GS-fed animals. These included early stage fatty degenerations, fatty degeneration around the portal triad, diffuse fatty degeneration, hepatic cords disruption and hepatic damage. The degenerative changes coupled with enhanced visceral adiposity could potentiate hepatic steatosis in the GS-fed rodents, while honey eaters enjoyed healthier growth. Despite the high fructose content of honey, it was absolved of culpability in all pathological changes observed in the GS-fed rats.

Pure natural honey is said to contain prebiotics which competitively activate beneficial intestinal bacteria flora in consumers[7,9], inhibit infectious agents[9,28], and enhance digestive functions[9]. Oligosaccharides cause a reduction of the flora pathogens, increase bifidobacteria (beneficial bacteria), and an increase of availability of minerals[9,10]. This facilitates metabolic activities and brings about healthy growth in honey eaters. In

addition to greater intestinal villi of high fibre diets of rodents, increasing the fibre content of the diets reduces the susceptibility to gastric infections[9]. This suggests a potential nutritional and health benefits of substituting honey for refined sugar such as cane syrup in the animal feed and by extension human diet.

It may be concluded that feeding of rodents with refined sugars such as cane syrup from neonatal age is associated with histopathological changes with consequent alterations in the organs' functions. Unlike GS, dietary supplementation with natural honey had positive influence on the visceral organs' growth and morphology, which could plausibly enhance their physiology. In conclusion, honey consumption is beneficial and could readily substitute for refined sugars such as dietary fructose. Nonetheless, there is a need to probe further to unravel the mechanisms behind the physiological benefits of honey.

Acknowledgement

This study was funded by the Tertiary Education Trust Fund (TETFUND), Abuja, Nigeria as a part of doctoral programme. The work was also supported by grants from National Research Foundation (NRF), South Africa; University of the Witwatersrand, South Africa; and the Faculty of Veterinary Science, University of Pretoria, South Africa. Thanks are also due to CAS staff and Ms. Margaret Badenhorst for technical assistance; and Mr. Jimmy Olusakin for reading the histological slides and taking the photomicrographs.

Conflict of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

1 Johnson R J, Segal M S, Sautin Y, Nakagawa T, Feig D I, Kang D, Gersch M S, Benner S & Sa´nchez-Lozada L G, Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease, *Am J Clin Nutr,* 86 (2007) 899.

2 Rutledge A C & Adeli K, Fructose and the metabolic syndrome: pathophysiologyand molecular mechanisms, *Nutr Rev* 65 (2007) S13.

3 Melanson K J, Zukley L, Lowndes J, Nguyen V, Angelopoulos T J & Rippe J M, Effects of high-fructose corn syrup and sucrose consumption on circulating glucose, insulin, leptin, and ghrelin and on appetite in normal-weight women, *Nutrition,* 23 (2007) 103.

4 Ludwig D S, Peterson K E & Gortmaker S L, Relation betweenconsumption of sugar-sweetened drinks and childhoodobesity: a prospective, observational analysis, *Lancet,* 357 (2001) 505.

5 Alvarez-Suarez J M, Tulipani S, Diaz D, Estevez Y, Romandini S, Giampieri F, Damiani E, Astolfi P, Bompadre S & Battino M, Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds, *Food Chem Toxicol,* 48 (2010) 2490.

6 Busserolles J, Mazur A, Gueux E, Rock E & Rayssiquier Y, Metabolic syndrome in the rat: females are protected against the pro-oxidant effect of a high sucrose diet, *ExpBiol Med,* 227 (2002) 837.

7 Busserolles J, Gueux E, Rock E, Mazur A & Rayssiquier Y, Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose, *J Nutr,* 132 (2002) 3379.

8 Gharzouli K, Amira S, Gharzouli A & Khennouf S, Gastro protective effects of honey and glucose-fructose-sucrose-maltose mixture against ethanol-, indomethacin-, and acidified aspirin induced lesions in the rat, *ExpToxicol Pathol,* 54 (2002) 217.

9 Ajibola A, Chamunorwa J P & Erlwanger K H, Nutraceutical values of natural honey and its contribution to human health and wealth, *Nutr Metab (Lond),*9 (2012) 61.

10 Rivero-Urgell M & Santamaria-Orleans A, Oligosaccharides: application in infant food (review), *Early HumDev,* 65 (2001) 43.

11 Pacha J, Development of intestinal transport functions in mammals, *Physiol Rev,* 80 (2000) 1633.

12 Pacha J, Vagnerova R & Bryndova J, Carbenoxolone accelerates maturation of rat intestine, *Pediatric Res,* 53 (2003) 808.

13 Strader A D & Woods S C, Gastrointestinal hormones and food intake, *Gastroenterology,* 128 (2005) 175.

14 Konturek SJ, Pepera J, Zabielski K, Konturek PC, Pawlik T, Szlachcic A & Hahn EG, Brain-gut axis in pancreatic secretion and appetite control, *J PhysiolPharmacol,* 54 (2003) 293.

15 Ajibola A, Idowu G O, Amballi A A, Oyefuga O H & Iquot I S, Improvement of some haematological parameters in albino rats with pure natural honey, *J Biol SciRes,* 2 (2007) 67.

16 Shambaugh P, Worthington V & Herbert J H, Differential effects of honey, sucrose, and fructose on blood sugar levels, *J Manip Physiol Ther,* 13 (1990) 322.

17 Leopold A S, Intestinal morphology of gallinaceous birds in relation to food habits, *J Wildl Manage,* 17 (1953) 197.

18 Guth P H, Paulsen G & Nagata H, Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat. Effect of prostaglandin cytoprotection, *Gastroenterology,* 87 (1984) 1083.

19 McAnuff M A, Omoruyi F O, Gardner M T, Morrison E Y & Asemota H N, Alterations in intestinal morphology of streptozotocin induced diabetic rats fed Jamaican bitter yam (*Dioscoreapolygonoides*) steroidal sapogenin extract, *Nutr Res,* 23 (2003) 1569.

20 Baltrop D & Brueton M J, The gastrointestinal tract and short term toxicity tests, *Short-term toxicity tests for non-genotoxic effects,* edited by PJ Bordeau, (John Wiley and Sons Ltd, New York, USA) 1990, 99.

21 Munro I C, Bernt W O, Borzelleca J F, Flamm G, Lynch B S, Kennepohl E, Bar E A & Modderman J, Erythritol: An interpretive summary of biochemical, metabolic, toxicological and clinical data, *FoodChemToxicol,* 36 (1998) 1139.

22 Erlwanger K H & Cooper R G, The effects of orally administered crude alcohol and aqueous extracts of African potato (*Hypoxis hemerocallidea*) corm on the morphometry of viscera of suckling rats, *Food Chem Toxicol,* 46 (2008) 136.

23 White J W & Doner L W, Honey composition and properties: Beekeeping in the United States, *Agric Handbook* 335 (1980) 82.

24 Sieck G C, Nance D M & Gorski R A, Regulation of feeding behaviour in the prepubertal female rat, *Physiol Behav,* 21 (1978) 727.

25 Satchithanandam S, Apler M V, Calvert R J, Leeds A R & Cassidy M, Alteration of gastrointestinal mucin by fibre feeding in rats, *J Nutr,* 120 (1990) 1179.

26 Yamauchi K E & Isshiki Y, Scanning electron microscopic observations on the intestinal villi in growing white leghorn and broiler chickens from 1 to 30 days of age, *Brit Poultry Sci,* 32 (1991) 67.

27 Yu B & Chiou P W S, Effects of crude fibre level in the diet on theintestinal morphology of growing rabbits, *Lab Animals,* 30 (1996) 143.

28 Kunz C & Rudloff S, Biological functions of oligosaccharides in human milk, *Acta Paediatr,* 82 (1993) 903.