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Therapeutic Diets for Diarrhea: Biological Evaluation in Rats

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Abstract: The present research dealt with the evaluation of the anti-diarrheal activity of two therapeutic diets in model of castor oil induced diarrhea in rats. Formula I contain modest amount of skimmed milk, formula II was lactose restricted diet. Both formulae contain cereals, legumes, honey and edible source having anti-diarrheal activity. The nutritional value of the two formulated therapeutic diets was evaluated in normal growing rats in comparison to reference formula, milupa special formula, in addition to control balanced diet (contain 10% protein supplemented from casein). The evaluation of nutritional value depended on determination of total food intake, body weight gain, food efficiency ratio and protein efficiency ratio. Nutritional status of rats fed different diets was also evaluated through determination of certain biochemical parameters such as percentage haematocrit, blood haemoglobin concentration, plasma total protein, albumin, iron, phosphorus, zinc, magnesium, retinol and ß-carotene. Results showed that anti-diarrheal activity of formula I was superior compared to formula II. Milupa and formula I have higher values of protein efficiency ratio and food efficiency ratio than control casein diet which were significant in case of Milupa. However diet II showed comparable values to control. Biochemical parameters showed higher values of plasma total protein, magnesium and retinol of rats fed Milupa diet. Feeding diet II produced significant increase of plasma iron magnesium and retinol. However, only significant increase of plasma magnesium has been observed when feeding diet I.

Key words: Therapeutic diets, diarrhea, rats

Introduction

Diarrhea, particularly persistent diarrhea is among the most important causitive factors of malnutrition (Boehm, *et al*., 1998). However, Javaid *et al.* (1991) reported that malnutrition itself contributes in the high incidence of diarrhea. So, it was of importance to formulate new therapeutic diets having both anti-diarrheal activity and simultaneously supplement the body with protein, energy and certain essential micronutrients needed during diarrheal episodes to improve the nutritional status. Micronutrients that have been postulated to have potential beneficial importance during diarrhea are zinc, vitamin A and folic acid (Fuchs, 1998; Gracey, 1999; Shoda *et al*., 2002). Plants constituents that have been reported to have anti-diarrheal effects are pectin, tannins and ß-carotene (Balbaa *et al*., 1981). Pectin has the property of conjugating toxins and enhancing the physiologic functions of the digestive tract through its physical, chemical and antibacterial properties. It is of value in the treatment of intestinal disorders such as diarrhea and dysentery. Pectic substances (Whistler and Smart, 1953) occur without exception in all higher plants. They are found most abundantly in the primary cell wall and in the intercellular layer, but secondary walls contain small amounts. Some plant juices are quite rich in these substances. Pectins are more abundant in fruits and roots. Carrot and soybean have been reported to contain pectin (Konno *et al*., 2002; Fransen *et al*., 2000). Carrot is rich in ß-carotene (Chindavijack *et al*., 1996) which is a precursor of vitamin A and has been reported to have a beneficial effect towards diarrhea (Gracey, 1999). Tannins present in *Ceratonia siliqua* and *Ciser arietinum* (Priolo *et al*., 2000; Al-Adawy, 2002) have been reported to have antibacterial and antiviral activity, thereby may have beneficial effects during infective diarrhea. *Cicer arietinum, Ceratonia siliqua*, carrot and rice have been used in Egypt since ancient times to treat diarrhea in Folk medicine. Some authors showed honey to have anti-diarrheal effect and to improve blood haemoglobin of anaemic patients (Hassan and Shawky, 1997). The anti-diarrheal effect of honey might be due to its anti-microbial activity (El-Fikky and Ibrahim, 1997) and through improvement of immune function (Melentkova *et al*., 1993).

The present research was postulated to make use of some of the previously mentioned edible plants such as *Cicer arietinum, Ceratonia siliqua*, carrot, rice and soybean in two formulae for infants with diarrhea, in addition of using honey as sweetener. One formula contained chicken meat as source of animal protein (lactose restricted formula), the other formula contained modest amount of skimmed milk and fortified with zinc and vitamin A.

The aim of the present research is to study the anti-diarrheal activity of the newly formulated therapeutic diets in rats. The aim includes evaluation of the nutritional

value of the therapeutic diets in rats through determination of body weight gain, protein efficiency ratio and food efficiency ratio with the assessment of different biochemical parameters reflecting nutritional status.

Materials and Methods

I. Biological Evaluation of the nutritional value of the therapeutic diets and the nutritional status of rats fed these diet

Materials

Therapeutic diets: In a previous work (Soliman *et al*., 2001) we have formulated and prepared two therapeutic diets for diarrhea, the constituents and chemical composition of which are present in Table 1. These diets were biologically evaluated in rats, in the present study. Milupa HN25 special formula (made in Germany by Milupa GmbH and Co, KG) was used as reference formula, its chemical composition is shown in Table 1 as written on packing. Some of the chemical constituents are not appeared on packing such as Fe, Zn and ash.

Experimental diets: Appropriate amounts of each of the previously prepared therapeutic diets and reference formula were taken so as to contain 10 g protein. The fat content was completed by corn oil to 10%, fibres were completed to 3% by cellulose. An amount of 3.5% salt mixture (Briggs and Williams, 1963), 1% water soluble vitamin mixture (the oil soluble vitamins were given weekly to rats separate from the diet) (Morcos, 1967) and 24.1 g% sucrose were added to each diet. The diets were completed by corn starch to 100%. A control diet containing 10% protein supplemented from casein was prepared. The composition of the different experimental diets are shown in Table 2.

Animals: White male and female albino rats of 50–60 g body weight were used in the present experiment. The animals were kept individually in wire bottomed cages at room temperature of 25±2°C.

Methods: This experiment was designed to evaluate the protein efficiency ratio of the formulated therapeutic diets in comparison to reference diet (milupa) and a casein diet as control. In addition of assessing their effect on body weight, food efficiency ratio and biochemical parameters reflecting nutritional status.

Rats were divided into 4 groups each of 8 rats. The rats of each group were fed one of the prepared experimental diets present in Table 2 for 4 weeks. During the period of the experiment, the food intake was calculated and the rats were weighed twice weekly. Growth curves were drawn representing the relationship between the rats body weight and time. After the end of experimental period, biological changes including food intake, body weight gain, food efficiency ratio (body weight gain/total food intake) and protein efficiency ratio (body weight gain/total protein intake) were calculated. Growth rates of rats of different groups were calculated as g/g body weight/day. At the end of experiment rats were fasted 16–18 h. The blood samples were drawn for determination of haemoglobin concentration (Betke and Savelsberg, 1956) and percentage haematocrit (Strumia *et al*., 1954). Plasma was separated from heparinized blood by centrifugation for determination of plasma total protein (Rheinhold, 1953), albumin (Doumas *et al*., 1972), iron (Tabacco *et al*., 1981), phosphorus (Gamst and Try, 1980), calcium (Gitelman, 1967), zinc (Homsher and Zak, 1985), magnesium (Gindler and Heath, 1971), retinol and ß-carotene (Neeld and Pearson, 1963). The results were statistically analysed using student's t-test.

II. Anti-diarrheal test (Iwao and Terada, 1962; Niemegeers *et al*., 1981)

Materials

- Rats: Female albino rats weighing 138.5 g±4.143 SE were used in the present experiment.
- Therapeutic diets: Formula I and formula II shown in Table 1.
- *Ceratonia siliqua* pods without seeds, reduced to very fine powder.
- Roasted *Cicer arietinum* reduced to very fine powder.
- Carrot, dried and grinded to fine powder.
- Castor oil.

Methods: Rats were used after overnight food deprivation. For the experiment the rats were housed in individual cages with no access to drinking water. Ratswere divided into two division, the first contain 2 group (gp_1, gp_2), the second contain 3 groups (gp_3 gp_4 gp_5). Each group included 6 rats. Four grams of either formula I or II (after adding very little equal amount of water) were given orally by gavage to each rat of gp_1 and gp_2 respectively. While, 2.5 g of *Ceratonia siliqua, Cicer arietinum* and carrot were given orally as previous to each rat of gp_3, gp_4 and gp_5 respectively. One hour later, 1 ml of castor oil was administered orally to each rat. Stools were collected on non wetting paper sheets of uniform weight up to 24 h after administration of the castor oil. Every 15 min during the first 8 h, urine is drained off by gravity and the net stool weight, termed early diarrheal excretion, was recorded. The diarrhea-free period is defined as the time between castor oil

Table 1: Chemical composition of the newly formulated therapeutic diets for diarrhea and reference formula (milupa) per 100 g

Parameters							Minerals (mg)								
Formula	Protein g	Fat g	Available carbohydrate g	Fibre g	Ash g	Moisture g	Fe	Ca	P	Mg	Zn	K	Na	Vitamin A (RE)*	Total energy Kcal.
Milupa	17.5	8.4	67.6	1.1	-	-	-	500	350	50	-	690	280	420	416
Formula I	21.0	4.5	64.7	2.43	2.7	4.5	4.9	188	345	90	7.4	635	51	305	383
Formula II	24.0	2.8	63.0	2.53	3.15	4.3	3.2	74	200	54	1.5	503	95	1020	373

*RE = Retinol equivalent

Ingredients of milupa: Lactose reduced skimmed milk powder, precooked rice, banana powder, maltodextrin, sodium caseinate, apple powder, vegetable fat, starch, calcium carbonate, vitamins and sodium chloride

Ingredients of formula I: Roasted *Cicer arietinum* (chick pea), soybean powder, precooked wheat, *Ceratonia siliqua* (Carob), skimmed milk (all the ingredients were dried and reduced to powder form), zinc sulphate, vitamin A and honey

Ingredients of formula II: Precooked chicken meat (breast without skin), soybean powder, precooked rice, carrot (all the ingredients were dried and reduced to powder form) and honey (lactose restricted formula)

Table 2: Composition of the different experimental diets (g/100 g diet)

Type of experimental diets				
Composition of the diets	Control	Diet I (from formula I)	Diet II (from formula II)	Milupa (reference diet)
*Composition of the amount taken from the formula:				
Protein		10.0	10.0	10.0
Fat		2.143	1.167	4.8
Carbohydrate		30.8	26.250	38.629
Fibre		1.157	1.054	0.629
Casein	10.3	-	-	-
Corn oil	10.0	7.857	8.833	5.2
Cellulose	3.0	1.843	1.946	2.371
Sucrose	24.1	24.1	24.1	24.1
Salt mixture	3.5	3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0	1.0
Corn Starch	Completed to 100	Completed to 100	Completed to 100	Completed to 100
Total	100	100	100	100
*Composition of : 47.619 g of formula I	42.667 g of formula II		57.143 g of milupa	

administration and the occurrence of the first diarrheal output. Stool occurring between 8 and 24 h after castor oil administration are called late diarrheal excretion. Evaluation of anti-diarrheal effect correlates with the decrease of 24 h stool output (Stool weight) and with increase of diarrhea-free period. Inhibitors of prostaglandin biosynthesis increase the diarrhea free period but do not affect early diarrheal secretion (Niemegeers *et al*., 1981).

Parameters of rats of gp_1 and gp_2 were compared with each other. Parameters obtained of rats of gp_3, gp_4 and gp_5 were compared with each others. Statistical analysis of student's t-test was applied to the results.

Results and Discussion

Table 3 showed that rats fed diet I showed significant increase in final body weight ($P < 0.05$), body weight gain ($P < 0.01$) and total food intake ($P < 0.001$) with remarkable increase in both protein efficiency ratio and food efficiency ratio compared with control. Feeding milupa diet produced significant increase in final body weight ($P < 0.05$), body weight gain ($P < 0.025$), food efficiency ratio ($P < 0.025$) and protein efficiency ratio ($P < 0.05$) with remarkable non significant increase in total food intake. Rats fed diet II showed significant increase in total food intake with non significant change in body weight gain, food efficiency ratio and protein efficiency ratio. Growth rates of rats were only significantly higher when feeding either milupa ($P < 0.01$) or diet I ($P < 0.001$) compared to control. Growth rate of rats fed diet II showed non significant change compared to control.

Although, protein efficiency ratio of milupa diet is higher than that of diet I and II and significantly higher than control casein, however the significant increase of total food consumed by rats of diet I and II may show important advantages when given to infants with diarrhea. Since those patients usually suffer anorexia (Fuchs, 1998) because this effect reflect the palatability of diet I and II which may show better taste than milupa.

Growth curves of rats (Fig. 1) showed that rats fed diet II showed gradual increase in body weight during the experimental period which did not show non significant change when compared to control casein i.e. comparable to casein diet. Growth curves of rats fed diet I also showed gradual increase in body weight which was significantly higher than control after 15 days, 21 days, 24 days and 28 days of the start of experiment. Milupa diet produced significant increase in body weight after 21 days, 24 days and 28 days compared to control casein.

Biochemical parameters (Table 4) showed that feeding milupa diet produced significant increase in plasma total protein ($P < 0.005$) which coincided with its significant

Table 3 : Nutritional parameters of different experimental groups. (Values are means±S.E)

Parameters / Type of diet	Initial body weight g	Final body weight g	Body weight gain g	Total food intake g	Growth rate g/g/day	Total protein intake g	Total fat intake g	Total carbohydrate intake g	Food efficiency ratio	Protein efficiency ratio
Formula I	58.4±1.49	115.6^{a}±3.05	57.3^{c}±2.12	379^{a}±9.78	0.035^{a}±0.001	37.9^{a}±0.978	37.9^{a}±0.978	274.8^{a}±7.086	0.151±0.006	1.52±0.067
Formula II	58.3±1.46	103.3±3.28	45.0±2.21	346^{a}±8.6	0.028±0.001	34.6^{a}±0.86	34.6^{a}±0.86	250.85^{a}±6.232	0.130±0.006	1.3±0.06
Milupa	58.3±1.42	116.3^{a}±3.19	58.0^{b}±2.57	337.1±12.12	0.036^{c}±0.002	33.7±1.21	33.7±1.21	244.4±8.789	0.172^{b}±0.009	1.72^{a}±0.101
Control	58.0±1.74	104.6±3.49	46.6±2.22	319.6±8.44	0.029±0.001	31.9±0.84	31.9±0.84	231.7±6.12	0.146±0.005	1.46±0.049

Values significantly differ from control : a : P < 0.05, b: P < 0.025, c : P < 0.01, e : < 0.001

Table 4: Biochemical parameters of rats of the different experimental groups (Values are means±SE)

Parameters / Type of diets	Control	Diet I	Diet II	Milupa
Haemoglobin (g dl^{-1})	10.4±0.442	11.2±0.543	10.1±0.599	9.0±0.810
Haematocrit	39.0±1.788	39±0.275	41±1.309	40.0±1.811
Plasma:				
Total protein (g dl^{-1})	7.6±0.288	7.7±0.248	7.8±0.295	8.8^{d}±0.192
Albumin (g dl^{-1})	4.7±0.121	4.9±0.298	5.0±0.272	4.9±0.127
Iron (µg dl^{-1})	207±10.670	197±10.661	242^{a}±10.1	199±13.6
Phosphorus (mg dl^{-1})	5.2±0.152	5.1±0.173	5.1±0.285	5.3±0.143
Calcium (mg dl^{-1})	9.4±0.305	9.2±0.475	9.6±0.394	10.1±0.361
Zinc (µg dl^{-1})	107.0±3.91	102±3.410	106±4.49	104±3.66
Magnesium (mg dl^{-1})	2.03±0.042	2.49^{d}±0.119	2.35^{a}±0.132	2.39^{d}±0.091
Retinol (µg dl^{-1})	43.0±3.526	47±3.035	52^{a}±2.01	59.2^{b}±5.243
ß-Carotene (µg dl^{-1})	33.0±3.824	33.4±1.517	34.0±0.778	45.8±5.967

Values significantly differ from control : a : P < 0.05, b : P < 0.025, d : P < 0.005, e : P < 0.001

Table 5: Parameters reflecting anti-diarrheal effect of formula I and II, *Ceratonia siliqua*, carrot and *Cicer arietinum* (Values are mean±SE)

Parameters / Groups	Diarrhea free period (hours)	Early diarrheal excrection (g)	Late diarrheal excretion (g)	Stool out put after 24 h (g)
Formula I	6.3***±1.078	0.405±0.265	0.925±0.175	1.33±0.174
Formula II	2.007±0.609	0.718±0.309	0.685±0.228	1.403±0.212
Ceratonia siliqua	3.863±1.336	0.702±0.437	1.41±0.466	2.112±0.406
Carrot	1.035±0.236	1.093±0.410	1.168±0.355	2.262±0.192
Cicer arietinum	5.182±3.782	1.12±0.434	0.492±0.208	1.612±0.416

Significant value: ***: P < 0.01

high protein efficiency ratio i.e. improve protein status which was not reflected by significant increase in albumin, so it may be reflected in globulin fraction. Significant increase in plasma magnesium and retinol were also noticed (P < 0.005 and P < 0.025 respectively).

Feeding diet II showed significant increase in plasma iron (P < 0.05) compared to control. Although diet I contain higher contents of iron than diet II, however diet II has a beneficial effect in elevating plasma iron than diet I which might be due to presence of tannins rich sources in diet I such as *Ceratonia siliqua* and chick pea (Priolo, *et al*., 2000 and Al-Adawy, 2002), that may hinder iron absorption (Roe, 1978) however, this did not produce any reduction on plasma iron of rats fed diet I which may be due to presence of honey that have been reported to improve iron status (Hassan and Shawky, 1997). However, the presence of *Ceratonia siliqua* and *Cicer arietinum* are essential as they have beneficial anti-diarrheal effect (Loeb, *et al*., 1989; Garcia and Harum, 1975).

Plasma retinol showed significant higher levels (P < 0.05) when diet II was fed. Significant higher levels of plasma magnesium have been noticed in rats fed diet I and II (P < 0.005 and P < 0.05 respectively). The increased magnesium level might have beneficial effects in infants with diarrhea since infants with chronic diarrhea are usually magnesium depleted (Mittal *et al*., 1990). No other significant changes have been noticed in other biochemical parameters.

The induction of diarrhea with castor oil results from the action of ricinoleic acid formed by hydrolysis of the oil (Iwao and Terada, 1962; Watson and Gordon, 1962). Ricinoleic acid produces changes in the transport of water and electrolytes resulting in a hyper-secretory response (Ammon *et al*., 1974). In addition to hyper secretion, ricinoleic acid sensitizes the intramural neurons of the gut, Recently Uchida *et al*. (2000) suggest the involvement of nitric oxide from nerves on the diarrhea induced by castor oil in rats i.e. nitric oxide is involved in the mechanism of castor oil-induced diarrhea.

Table 5 showed formula I more superior than formula II when testing anti-diarrheal activity in castor oil model in rats. This evaluation based on that diarrhea free period of rats given formula I (6.3±1.078 h) was significantly higher than that of formula II (2.007±0.609 h.) (P < 0.01), in

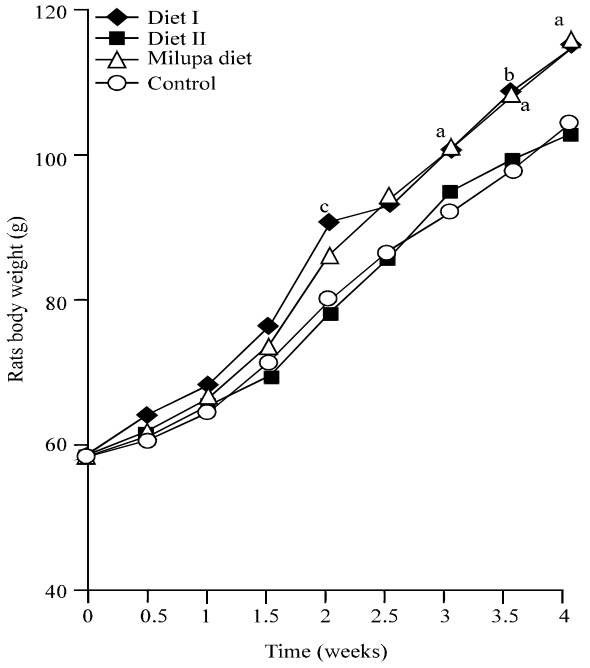

Fig. 1: Growth curves of the different experimental groups

Values are significantly differ from control
a: P<0.05 b: P<0.025 c: P<0.01

addition of early diarrheal excretion and stool out put after 24 h of rats given formula I were less than that of formula II. However, late diarrheal excretion is higher when formula I was given compared to formula II, which may refer to that the onset of anti-diarrheal activity of formula I was faster than formula II and that formula II had delayed onset of action.

When comparing rats given *Ceratonia siliqua*, carrot or *Cicer arietinum*, it was noticed that diarrhea free period was the highest and stool out put after 24 h was the least after giving *Cicer arietinum*, followed by *Ceratonia siliqua* then carrot i.e. *Cicer arietinum* was the best anti-diarrheal agent followed by *Ceratonia siliqua* then carrot. However, it was noticed that *Cicer arietinum* although superior as anti-diarrheal agent and though increased diarrheal free period, the early diarrheal excretion was high compared to rats given *Ceratonia siliqua* and carrot which may let us suggest that *Cicer arietinum* may have prostaglandin inhibiting activity as referred by Niemegeers *et al.* (1981). The presence of *Cicer arietinum* and *Ceratonia siliqua* in formula I which were superior as anti-diarrheal agent than carrot may reflect the superiority of formula I compared formula II that contain carrot.

The choice of the different ingredients of formula I (roasted *Cicer arietinum*, soybean, precooked wheat, *Ceratonia siliqua*, skimmed milk, zinc, vitamin A and honey) and formula II (Precooked chicken meat, soybean, precooked rice, carrot and honey) based on that most ingredients have been shown previously to have beneficial role towards diarrhea.

Sotelo *et al.* (1987) reported that both soy formula and chick pea proved promising in children with lactose intolerance with diarrhea, however diarrhea was better controlled by chick pea. In addition, chick pea flour has been used in the treatment of acute diarrhea of the infants by Garica and Harum (1975). Tannins in chick pea (Al-Adawy, 2002) may be one of the active constituents that have anti-diarrheal activity. However, Ouhida *et al.* (2002) reported that soybean is rich in pectin. Nakamura *et al.* (2001) proved that soybean soluble polysaccharides extracted from soybean cotyledons have a pectin-like structure. These constituent may be the cause of anti-diarrheal activity of soybean.

Mixed diets containing carrot flour and wheat flour versus lactose-free, soy protein formula showed comparable results in management of diarrhea (Alarcon, *et al.*, 1991). The presence of pectin in carrot (Messiaen and Van Cutsem, 1999) may be one of the cause of beneficial effect of carrot as anti-diarrheal agent. In addition carrot can replace enteral loss of sodium and potassium and contains sufficient glucose as free sugars to ensure optimum sodium and water absorption in the jejunum (Hascke *et al.*, 1980).

Tannins in carob pulb (Priolo *et al.*, 2000) may have the anti-diarrheal effect, however Sahle *et al.* (1992) reported decreased protein digestibility and food efficiency on inclusion of carob pod in meal. While in the present study, formula I that contain carob had food efficiency and growth rate more than casein control diet which might be due to presence of other constituent in the formula such as zinc, honey, skimmed milk and vitamin A. In spite of the reported disadvantage of carob, Loeb *et al.* (1989) proved that infants with acute diarrhea of bacterial and viral origin were treated using tannin-rich carob pod powder that normalized defecation and body weight and produced cessation of vomiting. It has been also reported that children of acute diarrhea given ORS + carob juice showed shorter duration, reduction of stool out put and ORS requirement compared to ORS and did not lead to any metabolic problems. Thereby carob juice has a role in the treatment of children's diarrhea (Serairi-Beji *et al.*, 2000). It has been also shown that successful preventive treatment of infantile diarrhea was verified by using formula containing semi-skimmed milk with 2% carob flour (Bustamante, 1970).

As a matter of fact, adherence of enterobacteria on intestinal epithelial cells is considered a major pathogenic

mechanism of infantile diarrhea. The cell free carbohydrate fraction of carrot soup and a 2% solution of carob were able to block hemagglutination and adherence of *Escherichia coli*. The active blocking agent was found in the oligosaccharide fraction of carob which may also explain the therapeutic effectiveness of carob as diarrheal remedy (Guggenbichler, 1983).

It has been reported that feeding full strength animal milk during diarrhea may have adverse effect (Bhutta *et al*., 1997). However, modest amount of skimmed milk with cereals reported to have beneficial role in management of diarrhea (Bhan *et al*., 1996). So we made use of this report in the present study to formulate formula I.

The use of honey as sweetener may have a role in management of diarrhea since honey contain fructose and glucose that readily absorbed without the need of disaccharidases that usually decreased during diarrhea (Khoshoo and Bhan, 1990). Honey also contain oligosaccharides that may stimulate activity of one or limited number of colonic bacteria that may improve the health (Gibson and Roberfroid, 1995).

Nutritional parameters showed that diet II was comparable to control casein and was better in elevating food intake. Diet I was superior to control and to diet II concerning food intake, body weight gain, growth rate and protein and food efficiency ratio.

The overall biochemical status reflected that diet I and II were comparable to casein diet and may be superior in elevating plasma iron, magnesium and retinol levels of rats fed diet II and concerning only magnesium when diet I was fed. The anti-diarrheal activity of formula I was superior to formula II. *Cicer arietinum* was the best anti-diarrheal agent followed by *Ceratonia siliqua* then carrot.

References

Al-Adawy, T.A., 2002. Nutritional composition and anti-nutritional factors of chick peas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods Hum. Nutr., 57: 83.

Alarcon, P., R. Montoya, F. Perez, J.W. Dongo, J.M. Peerson and K.H. Brown, 1991. Clinical trial of home available, mixed diets versus a lactose free, soy-protein formula for the dietary management of acute childhood diarrhea. J. Pediatr. Gastroenterol Nutr., 12: 224.

Ammon, H.V., P.V. Thomas and S. Phillips, 1974. Effects of oleic and ricinoleic acids on net jejunal water and electrolyte movement. J. Clin. Invest., 53: 374.

Balbaa, S.I., S. Hilal and A.Y. Zaki, 1981. Medicinal plant constituents, 3rd Edn. Egyptian Dar El-Kotob Cat. No. 4463 pages, 240: 355.

Betke, K. and W. Savelsberg, 1956. Styfenphotomatrisho hemoglobin estmmung mittels cyan-haemoglobin. Z. Biochem., 320: 431.

Bhan, M.K., N. Bhandari, S. Bhatnagar and R. Bahl, 1996. Epidemiology management of persistent diarrhea in children of developing countries, Indian. J. Med. Res., 104: 103.

Bhutta, Z.A., S.Q. Nizami and Z. Jsani, 1997. Lactose intolerance in persistent diarrhea during childhood the role of traditional rice-lentil (Khitchri) and yogurt diet in nutritional management. J. Pak. Med. Assoc., 47: 20.

Boehm, P., G. Nassimbeni and A. Ventura, 1998. Chronic non-specific diarrhea in childhood : How often is it iatrogenic ? Acta Paediatr, 87: 268.

Briggs, G.M. and M.A. Williams, 1963. A new mineral mixture for experimental rat diets and evaluation of other mineral mixtures. Fed. Proc., 22: 261.

Bustamante, P.S., 1970. Preventive treatment of infantile diarrheas with Milkobon (Semi-skimmed milk with 18% of milk group with added 2% of carob flour-Arobon). Minerva Pediatr., 22: 1332.

Chindavijack, B., C. Sornsuvit, O. Vajragupta and V. Pattamakom, 1996. Beta carotene serum profile after single dose administration of beta carotene capsule VS carrot. Thai. J. Phytopharm., 3: 7.

Doumas, B.T., W.A. Waston and H.G. Biggs, 1972. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta., 31: 87.

El-Fikky, A.A. and M. Ibrahim, 1997. Antimicrobial activity of honey : International symposium on Apitherapy, National Research Centre, Cairo, Egypt, March 8-9th, 1997.

Fransen, C.T., S.R. Haseley, M.M. Huisman, H.A. Schols, A.G. Voragen, J.P. Kamerling and J.F. Vliegenthart, 2000. Studies on the structure of lithium-treated soybean pectin : characteristics of the fragments and determination of the carbohydrate substituents of galacturonic acid. Carbohydr. Res., 328: 539.

Fuchs, G.J., 1998. Possibilities for zinc in the treatment of acute diarrhea. Am. J. Clin. Nutr., 68: 480S-3S.

Gamst, O. and K. Try, 1980. Determination of serum-phosphorus without deproteinization by ultraviolet spectrophotometry of the phosphomolybolic acid complex. Scand, J. Clin. Lab. Invest., 40: 483.

Garcia, S. and A. Harum, 1975. Use of chick-pea flour in the treatment of acute diarrhea of the infant Rev. Chil. Pediatr., 46: 319.

Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr., 125: 1401.

Gindler, E.M. and D.A. Heath, 1971. Colorimetric determination with bound calmagite of magnesium in human blood serum. Clin. Chem., 17: 662.

Gitelman, H.J., 1967. An improved automated procedure for the determination of calcium in biological specimens. Anal. Biochem., 18: 521.

Gracey, M., 1999. Nutritional effects and management of diarrhea in infancy. Acta Pediatr. Suppl., 88: 110.

Guggenbichler, J.P., 1983. Adherence of enterobacteria in infantile diarrhea and its prevention. Infection, 11: 239.

Hascke, F., G. Tsarmaklis, I. Steffan and F. Heresch, 1980. Carrot soup analysis, Wien Klin Wochenschr, 92: 57.

Hassan, A.R. and H. Shawky, 1997. Curative properties of some types of honey on common diseases in Egypt: International symposium on Apitherapy, National Research Centre, Cairo, Egypt, March 8-9th, 1997.

Homsher, R. and B. Zak, 1985. Spectrophotometric investigation of sensitivity complexing agents for the determination of zinc in serum. Clin. Chem., 31: 1310.

Iwao, I. and Y. Terada, 1962. On the mechanism of diarrhea due to castor oil. Jpn. J. Pharmacol., 12: 137.

Javaid, N., F. Haschke, B. Piesching, E. Schuster, C. Huemer, A. Shebaz, P. Ganesh, I. Steffan, R. Hurrel and M.C. Secretin, 1991. Interactions between infections, malnutrition and iron nutritional status in Pakistan, infants. A longitudinal study. Acta pediatric. Scant. Suppl., 374: 141.

Khoshoo, V. and M.K. Bhan, 1990. Intestinal glucoamylase and other disaccharidases in children with protracted diarrhea. Indian J. Med. Res., 92: 1.

Konno, H., S. Nakashima, T. Nakato and K. Katoh, 2002. Pectin-bound beta-galactosidase present in cell walls of carrot cells under the different calcium status. Physiol. Plant, 114: 213.

Loeb, H., Y. Vandenplas, P. Wursch and P. Guesry, 1989. Tannin-rich carob pad for the treatment of acute-onset diarrhea. J. Pediatr Gastroenterol Nutr., 8: 480.

Melentkova, V.M., N.M. Gracheva and G.P. Belikov, 1993. The chemoprophylaxis and chemotherapy of opportunistic infections. Antibiotiki, Ikhimioterapiia, 38: 77.

Messiaen, J. and P. Van Cutsem, 1999. Polyamines and pectins. II. Modulation of pectic-signal transduction. Planta, 208: 247.

Mittal, N.K., H.P. Sachdev and H.S. Yadav, 1990. Serum and rectal mucosal magnesium status in acute and chronic diarrhea. J. Trop. Pediatr., 36: 121.

Morcos, S.R., 1967. The effect of protein value of the diet on the neurological manifestations produced in rats by B-immunodipropionitrile. Brit. J. Nutr., 21: 269.

Nakamura, A., H. Furuta, H. Maeda, Y. Nagamatsu and A. Yoshimoto, 2001. Analysis of structural components and molecular construction of soybean soluble polysaccharides by stepwise enzymatic degradation. Brosci Biotechnol. Biochem., 65: 2249.

Neeld, Jr. J.B. and W.N. Pearson, 1963. Macro- and micromethods for the determination of serum vitamin A using trifluoroacetic acid. J. Nutr., 79: 454-462.

Niemegeers, C.J.E., F.C. Colpaert and F.H.L. Awouters, 1981. Pharmacology and anti-diarrheal effect of loperamide. Drug Dev. Res., 1: 1.

Ouhida, I., J.F. Perez and J. Gasa, 2002. Soybean (*Glycine max*) cell wall composition and availability to feed enzymes.

Priolo, A., G.C. Waghorn, M. Lanza, L. Biondi and P. Pennisi, 2000. Polyethylene glycol as a mean for reducing the impact of condensed tannins in carob pulp : effects on lamb growth performance and meat quality. J. Anim. Sci., 78: 810.

Rheinhold, J.G., 1953. Total protein, albumin and globulin in standard methods of clinical chemistry. D. Seligron, Ed. New York, Academic press, Inc., 1: 88.

Roe, D.A., 1978. Diet-Drug Interactions and incompatibilities: In Nutrition and drug Interrelations. Eds. Hathcock, J.N. and Coon, J. Academic Press, New York, pp: 319.

Sahle, M., J. Coleou and C. Haas, 1992. Carob pod (*Ceratonia siliqua*) meal in geese diets. Br. Poult. Sci., 33: 531.

Serairi, Beji, R., L. Mekki-Zouiten, L. Tekaya-Manoubi, M.H. Loueslati, F. Guemira and A. Ben Mansour, 2000. Can carob powder be used with oral rehydration solutions for the treatment of acute diarrhea. Med. Trop., 60: 125.

Shoda, R., D. Mahalanabis, K.N. Islam, M.A. Wahed, and M.J. Albert, 2002. Folic acid supplementation on red kidney bean-induced diarrhea and enteric bacterial translocation into mesenteric lymph nodes in rats : a pilot study. Acta. Paediatr., 91: 51.

Soliman, S.M., M. Abou Sekkien, T.H. Hamed, H.F. El-Behairy, M. El-Keiy and S.Y. Al-Okbi, 2001. Acute and chronic diarrhea in children. Dietary management and its impact on nutritional status. Med. J. Cairo Univ. Accepted in January, 2001 (In press).

Sotelo, A., M. Hernandez, J. Larracilla, M.L. Arenas and E. Palapa, 1987. Use of chickpea (*Cicer arietinum* L.) in non-dairy formulas. II. Nitrogen balance in children with lactose intolerance, fed with a formula based on chick pea and a commercial soybean product. Arch. Latinoam Nutr., 37: 468.

Strumia M.M., A.B. Sample and E.D. Hart, 1954. An improved micro-haematocrit method. Am. J. Clin. Pathol., 221: 1016.

Tabacco, A., E. Moda and Tarli, 1981. An improved, highly sensitive method for the determination of serum iron using chromazurol B. Clin. Chem. Acta., 114: 287.

Uchida, M., Y. Kato, K. Matsueda, R. Shoda, A. Muraoka, and S. Yamato, 2000. Involvement of nitric oxide from nerves on diarrhea induced by castor oil in rats. Jpn. J. Pharmacol., 82: 168.

Watson, W.C. and R.S. Gordon, 1962. Studies on the digestion, absorption and metabolism of castor oil. Biochem. Pharmacol., 11: 229.

Whistler, R.L. and G.L. Smart, 1953. Polysaccharide Chemistry, Academic Press Inc., New York, USA, pp: 163.