

PREPARATION AND EVALUATION OF TWO SPECIAL FOODS IN RATS WITH LIVER CIRRHOSIS

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SUMMARY: Two special diets for liver cirrhosis have been formulated (I and II), prepared in bakery form and evaluated in liver cirrhotic rats. Both diets contain protein of high Fisher ratio and oil rich in medium chain triglycerides. Proximate analysis of both formulas and their contents of amino acids and fatty acids were assessed. Special diets were evaluated in cirrhotic rats. Liver cirrhosis was induced in rats by intra-peritoneal injection of CCL₄ (at a dose of 0.2 ml/kg rat body weight/day for 3 consecutive days per week) for 10 weeks. Proximate analysis showed that % fat, protein and carbohydrates in formula I was 29.48, 19.3 and 35.2 and in formula II was 28.73, 19.1 and 38.1. Amino acids profile revealed that branched chain amino acids (BCAA) were 126.2 and 120 mg/g protein in formula I and II respectively. The Fisher ratio of formula I and II was 1.912 and 1.632 respectively. GLC analysis of fatty acids showed that the contents of medium chain fatty acids were 42 and 55.2% of total fatty acids in formula I and II respectively. Feeding cirrhotic rats on either of the special formula showed improved liver function reflected by significant reduction of the activity of transaminases (ALT and AST) and total bilirubin and the significant increase in total protein and albumin in plasma. There was also significant decrease in plasma level of malondialdehyde (MDA) indicating reduction in oxidative stress compared to control cirrhotic rats. Both formulas produced increase in body weight gain compared to control cirrhotic rats. In conclusion, both special formulas in the present study improved liver function and reduced oxidative stress in cirrhotic rats reflecting their potential beneficial use in patients with liver cirrhosis.

Key Words: Liver cirrhosis, special foods.

INTRODUCTION

Liver cirrhosis is a condition of severe liver damage which impairs its ability to function properly. Liver cirrhosis is associated with complex metabolic disorders that lead to a catabolic state. Mal-assimilation and loss of protein resulting in malnutrition that highly prevalent among patients with liver cirrhosis and loss of protein resulting in malnutrition (1). So, malnutrition especially protein-calorie malnutrition is very common in patients

with liver cirrhosis (2). Liver cirrhosis is associated with reduced energy intake and increased resting energy expenditure (3). Increased morbidity and mortality rates are usually encountered among those patients (4). It has been reported that branched chain amino acids supplementation is considered one of the important sources of protein to improve protein malnutrition in liver cirrhotic patients (5). Since they metabolized extrahepatic so they do not represent any load on the diseased liver (6). So, dietary formula with high Fisher ratio (branched chain amino acid/aromatic amino acids) is important to

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improve nutritional state in liver cirrhotic patients. Formula containing medium chain triglycerides (MCTS) oil is important for cirrhotic patients since medium chain triglycerides could be absorbed without bile (7,8) where the later is not easily produced by the scarred liver. Elevated oxidative stress has been reported in a variety of chronic liver diseases which arises from increased formation of oxygen free radicals along with deficiencies of antioxidant vitamins and reduced antioxidant enzymes' activities (9-11). So it might be of importance to incorporate antioxidant sources in the nutritional supplements for liver cirrhotic patients. The aim of the present research is formulation, preparation and chemical and biological evaluation of two special formulas in liver cirrhotic rats.

MATERIAL AND METHODS

Materials

Artichock (*Cynara scolymus*), flaxseed (*Linum usitatissimum*), black seed (*Nigella sativa*), skimmed milk, whey protein, honey, wheat flour, coconut oil, raisin, yeast and buffalo meat were purchased from local markets, Giza, Egypt. Defatted soybean was supplied from Agriculture Research Centre, Egypt.

Male Sprague Dawley rats with average body weight of 107.8 ± 1.026 g were used in the study. The animals were kept individually in wire bottomed stainless steel cages at room temperature. Water and food were given ad-libitum.

CCl_4 was purchased from (Sigma, USA) for induction of liver cirrhosis.

Methods

Flaxseed (Giza 8) was ground and defatted by petroleum ether (40-60°C) using Soxhlet apparatus, dried, reduced to very fine powder and sieved for inclusion in formula I.

The edible part of artichoke was dried and reduced to powder form to be included in formula II.

Buffalo meat was washed and pressure cooked in the least amount of water. The cooked meat was cut into small pieces and dried in air-circulated oven at 40°C till complete dryness, then reduced to powder and saved in freezer until used in the preparation of diet for feeding cirrhotic control rats.

Two special formulas (I and II) were prepared into two bakery products for liver cirrhotic patients. Ingredients of formula I were flaxseed, soybean, honey, wheat flour, raisin, skimmed milk, coconut oil and yeast. Ingredients of formula II were artichoke, black seed, whey protein, honey, wheat flour, raisin, coconut oil and yeast.

Buffalo meat and the two formulas were dried, powdered and sieved through 100-mesh sieve. The dried samples were analyzed for moisture, protein, fat, crude fiber and ash contents using standard AOAC procedure (12). Minerals content of for-

Table 1: Composition of different balanced diets (g per 100 g).

Ingredients	Balanced containing casein	Balanced diet containing buffalo meat
Casein	11.9*	-
Corn oil	10	6.83
Sucrose	19.5	19.5
Starch	49.1	47.62
Salt mix.	3.5	3.5
Vit. mix.	1	1
Fiber	5	5
Dry buffalo meat (16.55 g):		
Protein	-	10
Fat	-	3.173
Ash	-	0.578
Carbohydrate	-	2.768
Crude fiber	-	0.031

* 11.9 casein has been shown to contain 10 g protein when analyzed (12).

mula I and II (K, Na, Fe and Zn) were determined through atomic absorption technique (spectrophotometer, Varian spectr AA 220).

Two balanced diets appeared in Table 1 were prepared in the present study for feeding normal control rats and cirrhotic control rats. Salt mixture and vitamin mixtures were prepared according to Briggs and Williams (13) and Morcos (14), respectively. Oil soluble vitamins were given orally in a dose of 0.1 ml/rat per week. The first balanced diet prepared for feeding cirrhotic control rats contained casein as source of protein, the second contained buffalo meat as source of protein. For preparation of the second balanced diet an appropriate amount of dried buffalo meat was added so as the diet would contain 10% protein, the other constituents, fat, crude fibers and carbohydrates that were present in such amount were calculated. Corn oil, starch and fibers were added so as the different constituents would be equal to that of the first balanced diet as shown in Table 1. The prepared formula I and II were given to rats after baking without any additions.

Samples of formula I and II were prepared according to Spackman *et al.* (15) and subjected to analysis using an automatic amino acid analyzer (Hitachi L-8500; Hitachi Ind, Tokyo, Japan) for assessing the amino acids content. Samples of formula I and II were prepared for determination of fatty acids according to the method of Vogel (16) and analyzed by GLC under the following conditions: Stationary phase: 10% diethylene glycosuccinate packed column; oven temperature, 170°C; detec-

tor temperature, 300°C; injector temperature, 250°C; carrier gas, N₂; flow-rate, 30 ml/min; air flow-rate, 350 ml/min; H₂ flow-rate, 350ml/min; detector, FID; chart speed, 2 cm/min. Identification of the fatty acid methyl ester was carried out by direct comparison of their retention times with standard samples of the fatty acid methyl esters analyzed under the same conditions. Quantification was based on peak area integration.

Thirty male rats were divided into five groups, each comprised of 6 rats. Group one served as normal control where rats fed on balanced diet containing casein all over the study period (seventy days). Groups two and three were the cirrhotic control groups where rats were fed on balanced diet containing casein or buffalo meat respectively as source of protein all over the study period. The other two groups (4 and 5) were the test groups, where liver cirrhotic rats were fed on formula I and II respectively. Liver cirrhosis was induced in the rats of cirrhotic control group and the two test groups according to Blonde-Cynober *et al.* (17) by intra-peritoneal injection of CCL₄ (at a dose of 0.2 ml/kg rat body weight/day for 3 consecutive days per week) for 10 weeks. During the experiment, body weights and food intake were recorded weekly. At the end of the experiment; total food intake, body weight gain and food efficiency ratio were calculated. After elapse of experimental period, rats were fasted 16 h and blood samples were withdrawn from eye vein orbital in tubes containing heparin for separation of plasma for the determination of alanine transaminase (ALT) (18), aspartate transaminase (AST) (18), total bilirubin (19), total protein (20), albumin (21) and malondialdehyde (MDA) (22). Plasma globulin concentration was calculated from total protein and albumin.

Statistical analysis

The results obtained were expressed as the Mean \pm SE. Biochemical results of rats of cirrhotic control group fed on balanced diet containing casein or buffalo meat were compared with normal rats using Student's t-test. Biochemical parameters of different groups of cirrhotic rats were compared with each other statistically using the one-way ANOVA analysis followed by the Duncan's test. T-student's test was applied to nutritional parameters of all the studied groups.

RESULTS

Proximate analysis of the special formulas

Chemical composition of the formulas shown in Table 2 clarified that both formulas had high percentage of protein. Protein provided 15.99% and 15.68% of calories from formula I and II respectively. It can be seen also that fat content was 29.5% and 28.7 % i.e. 54.92% and 53.08% of calories were supplemented from fat in formula I and II respectively. Carbohydrate contents

were 35.2% and 38.1% in formula I and II where they provide 29.09 and 31.25% of calories, respectively. Very low percentage of crude fibers was present in both formulas. The ash which reflects mineral contents were 1.58 and 1.49g/100g fresh sample of formula I and II respectively. K, Na, Fe and zinc contents were 983.15, 31.79, 2.1 and 18.26 mg respectively/100g fresh sample in formula I and 961.65, 28.22, 1.44 and 18.73 /100 g fresh sample in formula II respectively.

Amino acids

Amino acids profile of the two formulas is present in Table 3. The results showed that cystine was the lowest 1.21 mg/g protein, while glutamic was the highest 252 in formula I. In formula II the lowest amino acid was methionine 8 mg/g protein and the highest was glutamic 213.5. The Fisher ratio of formula I and II was calculated to be 1.912 and 1.632 respectively. The results revealed that both formulas are rich in BCAA (leucin, isoleucin and valine) which were 126.2 and 120 in formula I and II respectively.

GLC analysis of fatty acid methyl esters

The different fatty acid methyl esters of the formulas are shown in Table 4. It can be seen that fatty acid C12, medium chain saturated fatty acid, was the major fatty acid in formulae I and II (37.9% and 42.1% respectively),

Table 2: Chemical composition of fresh special formulas.

Ingredients/ 100g fresh sample	Formula I	Formula II
Moisture (g)	13.8	12.4
Protein (g)	19.3	19.1
Fat (g)	29.5	28.7
Ash (g)	1.58	1.49
Crude fibers (g)	0.70	0.22
Carbohydrate*	35.14	38.10
Calories	483.10	487.2
K (mg)	983.15	961.65
Na (mg)	31.79	28.22
Zn (mg)	18.26	18.73
Fe (mg)	2.10	1.44

* Calculated by differences .

while linoleic acid (C 18:2) was the major polyunsaturated fatty acid (10.3% and 12.5 respectively). Linolenic (C 18:3) is present only in formula II. The results revealed that both special formulas as expected are rich in medium chain fatty acids (42 and 55.2% of total fatty acids in formula I and II respectively). Medium chain fatty acids are C6, C8, C10 and C12.

Biological evaluation of the special formulas

Table 5 showed the biochemical parameters of different experimental groups. Cirrhotic control rats fed on balanced diet containing either casein or buffalo meat showed significant increase ($p < 0.001$) in AST, ALT, total bilirubin and MDA and significant decrease in while total protein, albumin and globulin ($p < 0.001$) when compared with normal control rats fed balanced diet containing casein.

ANOVA and Duncan's test showed that cirrhotic rats fed on balanced diet containing casein or either of the special formula improved liver function reflected in the significant reduction of the activity of plasma ALT, AST and total bilirubin ($p < 0.05$) and significant increase in total protein, albumin and reduced oxidative stress significantly through significant reduction in plasma MDA ($p < 0.05$) compared to cirrhotic rats fed balanced diet containing buffalo meat. Plasma globulin was significantly elevated in test groups and the group of rats fed balanced casein diet compared to cirrhotic rats fed balanced diet containing buffalo meat ($p < 0.05$). There was no significant difference in the biochemical parameters between the groups of liver cirrhotic rats fed formula I, II or balanced casein diet.

Table 6 showed the nutritional parameters of normal and liver cirrhotic rats. Liver cirrhotic control rats (fed casein or buffalo meat diet) showed significant reduction in all nutritional parameters compared with normal rats fed casein diet. Nutritional parameters of cirrhotic rats fed formula I showed significant increase in body weight gain and total food intake ($p < 0.05$), while feeding formula II to cirrhotic rats produced only significant increase in body weight gain ($p < 0.05$) compared to cirrhotic rats fed balanced buffalo meat diet. Feeding cirrhotic rats on balanced diet containing casein showed

Table 3: Amino acids (mg/g protein) profile of the special formulas.

Amino Acids	Formula I	Formula II
Theronine	38	52
Cystine	1.21	9
Methionine	5	8
Valine	32.7	36.5
Isoleucine	52	52
Leucine	41.5	31.5
Tyrosine	33	48
Phenylalanine	33	25
Lysine	20	26.5
Aspartic	75	83.5
Serine	28	31
Glutamic	252	213.5
Proline	61	34
Glycine	48	54
Alanine	42	46
Histidine	16.5	26
Arginine	46	98.5
Fisher ratio	1.912	1.632

significant increase in final body weight, body weight gain and total food intake ($p < 0.001$) compared to cirrhotic rats fed balanced buffalo meat diet.

DISCUSSION

The ingredients of the prepared special foods have been selected so as to contain appreciable levels of branched chain amino acids or high Fisher ratio,

Table 4: GLC analysis of fatty acids of the special formulas (as percentage of total fatty acids).

Fatty acids	Formula I	Formula II
C6	1.3	1.4
C8	-	6.3
C10	2.8	5.4
C12	37.9	42.1
C14	20.6	17.7
C16	11.4	10.6
C18:1	2.7	-
C18:2	10.3	12.5
C18:3	-	3.5
% Medium chain fatty acids	42	55.2

Table 5: Biochemical parameters of different experimental groups.

Plasma parameters	Groups				
	Normal control (casein)	Cirrhotic control (casein)	Cirrhotic control (buffalo meat)	Formula I	Formula II
AST (U/L) % Change	141.5 ± 0.764	166.5 ^b ± 1.176 -12	189 ^a ± 0.937 34	166 ^b ± 1.527 -12	164.5 ^b ± 1.118 -13
ALT (U/L) % Change	54.5 ± 0.764	65 ^b ± 1.154 -10	72.2 ^a ± 0.763 32	64.5 ^b ± 1.176 -11	66.5 ^b ± 0.764 -8
T. bilirubin (mg/dl) % Change	0.299 ± 0.002	0.357 ^b ± 0.010 -14	0.415 ^a ± 0.005 39	0.336 ^b ± 0.004 -19	0.342 ^b ± 0.002 -18
T. protein (g/dl) % Change	7 ± 0.058	5.9 ^b ± 0.070 16	5.1 ^a ± 0.060 -27	5.8 ^b ± 0.098 13	5.8 ^b ± 0.058 13
Albumin (g/dl) % Change	3.767 ± 0.042	2.95 ^b ± 0.062 7	2.767 ^a ± 0.029 -27	2.9 ^b ± 0.056 7	2.9 ^b ± 0.031 6
Globulin (g/dl) % Change	3.232 ± 0.056	2.92 ^b ± 0.031 26	2.32 ^a ± 0.056 -28	2.8 ^b ± 0.056 22	2.85 ^b ± 0.022 23
MDA (nmol/l) % Change	6.967 ± 0.128	16.3 ^b ± 0.228 -14	18.91 ^a ± 0.250 171	16.2 ^b ± 0.206 -14	16.75 ^b ± 0.138 -11

Values significantly differ from normal control (casein diet): *p<0.001

Data with different superscripts in the same row are significantly different (p<0.05) according to ANOVA and Duncan.

medium chain triglycerides and antioxidants. The special foods were formulated to have good amount of protein and high calories to improve malnutrition state for those patients of mild and moderate cases of liver cirrhosis but not for advanced cases. Those liver cirrhotic patients needs establishment of positive nitrogen balance (23). So, the present special foods have been formulated to contain appreciable percentage of protein with high Fisher ratio (1.9 and 1.6 for formula I and II respectively) referring to their suitability for liver cirrhotic patients, in order not to develop encephalopathy. On the other hand, it has been reported that patients with chronic liver disease have increased energy expenditure (24) and that most energy utilized in liver cirrhotic patients is derived from fat (25). So, appreciable amount of fat as medium chain triglycerides oil was added in the formulas to elevate the calorific contents. Medium chain triglycerides absorbed without the need of bile and it helps absorption of long chain fatty acids and oil soluble vitamins (26, 27). So it can improve utilization of fat and fat-soluble vitamins in liver cirrhotic patients.

As expected, liver dysfunction was noticed in liver cirrhotic rats reflected by the significant increase in the

activities of plasma AST, ALT and total bilirubin. As a matter of fact, the severity of liver synthetic dysfunction is estimated by measuring bilirubin (6). The significant decrease of plasma albumin and total protein in cirrhotic control rats noticed in the results clarified liver synthetic dysfunction of protein. Also cirrhotic control rats showed significant reduction in all nutritional parameters compared with normal rats reflecting malnutrition state as reported in liver cirrhotic patients (2).

Feeding liver cirrhotic rats the special formulas showed significant reduction in plasma activity of AST and ALT and total bilirubin with significant elevation of plasma total protein and albumin reflecting improvement of liver function. Also the results showed an improvement in the nutritional status of liver cirrhotic rats fed on formula I or II which was noticed in the significant increase of body weight gain together with plasma albumin. This may be due to presence of appreciable percentage of BCAA which was reported previously to improve plasma amino acids imbalance as well as protein metabolism in patients with liver cirrhosis (28), decreased frequency of complications of cirrhosis and improved nutritional status (29). BCAA supplementation improves hypoalbuminemia in decompensated cirrhotics (30).

Table 6: Nutritional parameters of different experimental groups.

Parameters	Groups				
	Normal control	Cirrhotic control (casein)	Cirrhotic control (buffalo meat)	Formula I	Formula II
Initial body weight (g)	108 ± 1.211	107.8 ± 0.601	107.3 ± 0.428	107 ± 1.527	107.7 ± 2.616
Final body weight (g)	207.3 ± 4.659	133 ^a ** ± 0.894	119.3 ^a ± 0.843	126 ± 3.872	125 ± 3.245
Body weight gain (g)	99.3 ± 5.029	25.2 ^a ** ± 0.749	11.8 ^a ± 0.601	19* ± 2.804	17.3* ± 1.706
Total food intake(g)	754.3 ± 3.392	673.8 ^a ** ± 3.113	637.2 ^a ± 2.761	651.3* ± 5.583	643.2 ± 3.059
Food intake (g/d)	10.8 ± 0.048	9.6 ^a ** ± 0.044	9.1 ^a ± 0.039	9.3 ± 0.082	9.2 ± 0.045
Food efficiency ratio	0.133 ± 0.006	0.037 ^a ± 0.001	0.019 ^a ± 0.009	0.029 ± 0.004	0.027 ± 0.003

Values significantly differ from normal control: ^a p<0.001

Values significantly differ from cirrhotic control buffalo meat: * p<0.05, ** p<0.001.

The two special formulas in the present study contain rich sources of BCAA. Formula I contains soybean and flaxseed. Soybean protein has been reported to contain high level of branched chain amino acids and Fisher ratio where Fisher ratio is 2.1 which provides the desirable level required in diet formulations for patient with chronic liver disease (31). Flaxseed protein is high in BCAA and Fischer ratio (31). Flaxseed protein fractions are rich in BCAA and of high Fischer ratio which suit liver diseases patients (32). Also flaxseed consumption conferred greater protection towards liver disease due to presence of lignans (33).

Whey protein was used as source of BCAA in formula II since it was cited previously to contain high level of branched chain amino acids (34). Previously it has been reported that whey protein-containing diet clearly suppressed the increased activity of plasma ALT and AST, the level of total bilirubin, histopathological signs of portal fibrosis, duct proliferation and prevascular sclerosis (35). So, supplementation of formula II with whey protein can have beneficial effect towards liver cirrhosis.

High oxidative stress in chronic liver disease demonstrated previously by Sumida *et al.* (11) was in the agreement of the present study where significant elevation of MDA was noticed in cirrhotic rats compared to control. It has been reported previously that the increase in the oxidation of fats and proteins is the most important mechanism in prevalence of energy protein malnutrition in liver cirrhotic patients (36). This oxidation might be

due to elevated oxidative stress in those patients. Feeding either of the formulas in the present study reduced oxidative stress represented by MDA.

Antioxidant phytochemicals in grains and fruits have obtained great attention for their potential roles in human disease. Phenolics were considered as a major group of compounds that contribute to this antioxidant activity. We have made use of this property in using dried fruits (raisin), vegetables (artichoke) and grains that contain antioxidant component in the present formulas (37-40). Also the presence of flaxseed that contain lignan, a flavonoidal compound, in formula I may render it extra antioxidant activity.

Honey used as sweetner in the formulas is also considered as a source of antioxidants (41) which may work in synergism with other antioxidants in the formulas (42).

Artichoke and *Nigella sativa* have been used as ingredients of the special formula II since they have been reported previously to have beneficial effect towards cirrhotic patients (43, 44). Artichoke extract has been shown to prevent oxidative stress-induced hepatotoxicity in rats (45). The reduction in MDA levels as indicator of lipid peroxidation noticed in cirrhotic rats fed either of the formulas may be due to presence of the different antioxidant sources in each formula.

It is worthy to mention that in the present research, the level of different minerals in the prepared formulas, met the needs of liver cirrhotic patients due to low level of Na and sufficient level of K, Zn and Fe.

It can be noticed that the sources of protein is important when dealing with liver cirrhosis, this is demonstrated by the different effect of balanced diets when contain either casein or buffalo meat as source of protein. Whereas balanced diet containing casein improved the liver cirrhotic state, that containing buffalo meat worsen it. The improvement shown by casein diet agreed with the previous work of El-Sayed *et al.* (46).

In conclusion, both special formulas in the present study improved liver function, malnutrition and reduced oxidative stress in liver cirrhotic rats reflecting their potential beneficial use in patients with liver cirrhosis. The present study demonstrated the beneficial effect of casein and the bad effect of buffalo meat towards liver cirrhosis.

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