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EVALUATION OF POTENTIAL HEALTH BENEFITS OF TWO NOVEL SPECIAL FOODS IN LIVER CIRRHOTIC PATIENTS

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Two therapeutic diets have been formulated (A & B) and prepared in bakery form for patients with liver cirrhosis. The formulation was based on the presence of protein of high Fisher ratio and fat rich in medium chain triglycerides. Both formulas contained edible sources of antioxidants. Honey was added as a sweetener. Proximate analysis of Formula B and its contents of amino acids and fatty acids were carried out. Formula A was analysed in previous research for the same above mentioned composition. The two formulas were sensory evaluated by liver cirrhotic patients. Potential benefits of both formulas in addition of nutritional advice were evaluated in liver cirrhotic patients. Biochemical parameters reflecting liver function (plasma AST, ALT, ALP, total bilirubin, direct bilirubin and ammonia), oxidative stress (plasma NO and MDA) and nutritional status (plasma total protein, albumin, globulin and A/G ratio) were studied before and after two months of dietary intervention. The nutritional status of patients was evaluated through anthropometric measurements, food intake and selected biochemical parameters. Proximate analysis results showed that formula B contain 17.6% protein and 7.5% fat. Amino acids analysis of the same formula showed that its Fisher ratio was 2.49. GC analysis of fatty acids revealed that medium chain fatty acids constitute 38.6% of total fatty acids. The results of sensory evaluation showed that overall score of formula A was significantly higher than that of formula B. Nutritional status determined through triceps skin fold at the start of the clinical study showed 38% of cases were normal, 8% were severely malnourished and 54% over normal. Analysis of mean dietary intake of patients in the beginning of the study revealed that all liver cirrhotic patients were hypo-caloric. All estimated nutrients were lower than RDA except for protein. Mean dietary intake of patient after two months of the study revealed that all patients increased their caloric intake however they were still hypo-caloric. Comparing biochemical parameters of patients before and after dietary intervention of either formulas and nutritional advice revealed that all parameters reflecting liver dysfunction were non significantly improved. However AST activity significantly decreased on supplementation of diet A. A significant increase in plasma albumin and total protein was noticed in patients after both dietary interventions which may reflect some improvements in liver synthetic function and nutritional status. Ammonia was only significantly decreased in patients given formula A. Nitric oxide and MDA were significantly reduced in both dietary interventions with different degrees.

In conclusion, both dietary interventions in addition of dietary advice in the present study have beneficial effects towards liver cirrhotic patients concerning reduction of oxidative stress and inflammation. Formula A was superior in reducing plasma AST activity and ammonia level.

INTRODUCTION

Liver cirrhosis is a condition of severe liver damage which impairs its ability to function normally. Liver cirrhosis is associated with complex metabolic disorders and regularly leads to a catabolic state. Catabolism, mal-assimilation and loss of protein are the main reasons underlying reduced nutritional status frequently encountered in these patients [Mörk, 2007]. So, malnutrition especially protein-calorie malnutrition is very common in patients with liver cirrhosis [Gundling & Schepp, 2008]. Protein intake that can be tolerated by patients with liver cirrhosis varies considerably [Lieber, 1999]. Patients with liver cirrhosis tend to be hypermetabolic and higher than normal supply of dietary protein is needed to achieve nitrogen balance. Most patients tolerate a normal or even increased dietary protein intake without risk of encephalopathy. In few patients intolerant to the required protein intake, branched chain amino acids supplements can provide the necessary nitrogen intake without detrimental effect on the mental state, perhaps even improving it [Marchesini *et al*., 2000; Laviano *et al*., 2005]. Branched chain amino acids are metabolized extrahepatic so they do not represent any load on the diseased liver [Molleston, 1997]. So dietary formulas with high Fisher ratio (branched chain amino acid / aromatic amino acids) are important to improve both the nutritional and health status of liver cirrhotic patients. Formula containing medium chain triglycerides (MCTS) oil may have potential benefits for cirrhotic patients since the scarred liver cannot produce bile easily. Medium chain triglycerides could be absorbed without bile [Mobarhan, 2000; Monsen, 1999]. MCTS can also enhance the absorption of both essential fatty acids and oil soluble vitamins that are usually mal-absorbed in such patients [Molleston, 1999]. The antioxidant vitamin E is among those mal-absorbed vitamins. 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 [Britton & Bacon, 1994; Kalra

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et al., 1994; Sumida *et al*., 2003]. So it might be of importance to incorporate antioxidant sources in the nutritional supplements for liver cirrhotic patients.

The aim of the present research was to formulate, prepare and study the potential benefit of two special formulas, in compensated liver cirrhotic patients with virus C on both health and nutritional status.

MATERIALS, SUBJECTS AND METHODS

MATERIALS

Cynara scolymus, *Nigella sativa*, *Cicer arietinum*, *Glycine max*, casein, corn oil, whey protein, honey, wheat flour, dried fruits, branched chain amino acids, coconut oil and yeast were purchased from Agriculture Research Centre and local markets, Egypt.

SUBJECTS

The subjects under study were thirteen male patients of compensated liver cirrhosis (mild and moderate cases) with virus C. Their age ranged from 32 to 65 years old. Ten male healthy subjects of matched age were studied as control.

METHODS

Preparation of the two special formulas

Two special formulas (A & B) were prepared into two bakery products for liver cirrhotic patients. Ingredients of formula A were *Cynara scolymus*, *Nigella sativa*, whey protein, honey, wheat flour, raisin, coconut oil and yeast. Ingredients of formula B were *Cicer arietinum*, *Glycine max*, casein, corn oil, honey, wheat flour, dried dates, branched chain amino acids, coconut oil and yeast.

Chemical analysis of formula B

Moisture was determined in special formula B then it was dried, powdered and sieved through 100-mesh sieve for analysis of protein, fat, crude fiber and ash according to standard AOAC procedure [AOAC, 1995]. Minerals content (K, Na, Fe and Zn) was determined using atomic absorption spectrophotometer (Varian spectr AA 220).

Determination of amino acids and fatty acids content of special formula B

A sample from formula B was prepared according to Spackman *et al*. [1958] and subjected to analysis using an automatic amino acid analyser (Hitachi L-8500; Hitachi Ind., Tokyo, Japan). Fatty acids were determined according to the method of Vogel [1961] and analysed by GLC under the following conditions: stationary phase: 10% diethylene glycosuccinate packed column; oven temperature, 170°C; detector temperature, 300°C; injector temperature, 250°C; carrier gas, N_2; flow-rate, 30 mL/min; air flow-rate, 350 mL/min; H_2 flow-rate, 350 mL/min; detector, FID; Chart speed, 2 cm/min. Identification of the fatty acids was carried out by direct comparison of their retention times with standard samples analysed under the same conditions. Quantization was based on peak area integration.

Proximate analysis, fatty acids, amino acids and minerals contents of formula A were determined in a previous research [Mohamed & Al-Okbi, 2009].

Sensory evaluation

Just after baking, the two formulas were cooled to room temperature and subjected to sensory evaluation by ten liver cirrhotic patients [Meiselman, 1978]. Each patient was asked to assign scores on a nine-point scale for appearance, crumb colour, texture, taste and overall acceptability. A sensory score of 5 or above was deemed acceptable, and a sensory score below 5 was considered unacceptable.

Design of the clinical study (intervention study)

Patients with liver cirrhosis were divided into two groups. Group one comprised seven patients; each patient was given daily quantity of 75 g from formula (A). Group two comprised six patients, each patient received daily amount of 75 g from formula (B). The study continued for 2 months. Nutritional status of all liver cirrhotic patients was assessed through anthropometric measurements and dietary intake. Biochemical analysis of blood was carried out at the start and at the end of the study.

Anthropometric measurements

Measurements of anthropometric parameters; body weight, height, mid arm circumferences (MAC) and triceps skin fold (TSF) were carried out. Arm muscle circumference (MC) and body mass index (BMI) were calculated according to Frisancho [1974] and George [1998] respectively: BMI (kg/m^2) = Weight / Squared Height, whereas MC (cm) = MAC (cm) – [TSF (mm) X 0.314].

At the start of the clinical study (before dietary supplementation), patient's nutritional status was assessed on the basis of BMI according to George [1998]. MAC, TSF and MC were compared with international standards. Nutritional status were again determined according to MAC, TSF and MC (70-80% of standard were moderately malnourished, 80--90% mild, >90% normal, > standard were overweight and obese).

Food intake

Patients were subjected to questionnaire for one-day dietary recall, in addition to frequency of food items consumed to determine the daily nutrient intake of patients. Analysis of intake of protein, fat, carbohydrates, calories, zinc, copper, iron and vitamin E and C per day was carried out using the computer program (World Food Dietary Assessment). The adequacy of nutrient intake was evaluated as percent of RDA [FAO/WHO, 1989]. After taking questionnaire, patients were advised to exclude meat, meat products, canned and frozen food and juices from their diets, reduce their intake of salt and salted foods, increase intake of skimmed milk and milk products, increase intake of fresh fruits, fresh vegetables, whole cereals and cereal products. At the end of the study another questionnaire for one-day dietary recall was taken from all patients. Mean dietary intake of patients at the end of the study was compared with that at the start.

Biochemical analysis of blood

Blood samples were obtained from fasted subjects. The blood samples were mixed with heparin for separation of plasma and determination of glucose [Trinder, 1969], ammonia [Gips & Wibbens-Alberts, 1968], total and direct bilirubin [Gambino, 1965], total protein [Rheinhold, 1953], albumin [Doumas *et al*., 1972] and activity of aspartate transaminase (AST) [Reitman & Frankel, 1957], alanine transaminase (ALT) [Reitman & Frankel, 1957] and alkaline phosphates (ALP) [Kochmar & Moss, 1976]. Globulin and albumin/globulin ratio were calculated. Plasma Nitric oxide (NO) [Monttgomery & Dymock 1961] and malondialdehye (MDA) [Satoh, 1978] were determined as indicators of inflammation and lipid peroxidation, respectively. The biochemical parameters of patients at the start of the clinical study were compared with those of the healthy control. Also, biochemical parameters of patients were compared before and after dietary supplementation. Statistical analysis of Student's t-test (2-tailed) was applied.

RESULTS AND DISCUSSION

Proximate analysis of the special foods

Proximate composition of formula B shown in Table 1 clarified that it contained 17.6% protein and 7.5% fat. Carbohydrate content was 57.1% that provides 62.32% of the calories. Protein and fat provided 19.24% and 18.44% of calories, respectively. Formula B can provide 366.25 calories/100 g. Ash, fiber and moisture contents were estimated to be 2.0%, 0.1% and 15.7%, respectively. K, Na, Fe and zinc contents were 1353, 50.6, 2.1 and 20.3 mg/100 g fresh sample, respectively. From previous work [Mohamed & Al-Okbi, 2009], the protein and fat content of formula A was 19.1% and 28.7%, respectively. Formula A can provide 487.2 calories per 100 g. K, Na, Fe and zinc contents of formula A were 961.65, 28.22, 1.44 and 18.73 mg/100 g fresh sample, respectively. These specific minerals were determined since in certain stage of liver cirrhosis as in case of ascites, Na intake must be reduced. Fe is needed in the presence of internal hemorrhage. Zinc is important for activity of antioxidant enzymes.

TABLE 1. Proximate composition and minerals in formula B (per 100 g fresh sample).

Ingredients	Per 100 g fresh sample
Moisture(g)	15.7
Protein (g)	17.6
Fat (g)	7.5
Ash (g)	2.0
Fiber (g)	0.1
Carbohydrate*(g)	57.1
Calories	366.3
K (mg)	1353.0
Na (mg)	50.6
Fe (mg)	2.1
Zn (mg)	20.3

* Calculated by difference

Amino acids

Amino acids profile of formula B is present in Table 2. The results showed that Fisher ratio of formula B (leucin + isoleucin + valine / phenylalanine + tyrosine) was calculated as 2.49. Formula A from previous work [Mohamed & Al-Okbi, 2009] showed Fisher ratio of 1.63.

GLC analysis of fatty acids

The fatty acids in formula B are shown in Table 3. It can be seen that lauric acid (C12) was the major medium chain fatty acid in the formula (28.4%), while linolenic acid (C18:3) was the major polyunsaturated fatty acid (18.4%). Stearic (C18:0) and oleic acid (C 18:1) were present as 3.2 and 13.6%, respectively. The results revealed that the special formula as expected is rich in medium chain fatty acids that accounts for 38.6% of total fatty acids. Medium chain triglycerides were absorbed rapidly because they do not require lipolysis or micelles for absorption. From previous work [Mohamed & Al-Okbi, 2009] the other formula (A) showed also high percentage of medium chain fatty acids (55.2%).

TABLE 2. Amino acids profile of formula B (g/100 g dry sample).

Amino acids	Formula B
Aspartic	0.04
Theronine	0.74
Methionine	0.39
Valine	1.05
Isoleucine	1.02
Leucine	1.52
Tyrosine	0.58
Phenylalanine	0.86
Lysine	1.05
Serine	0.07
Glutamic	3.85
Proline	0.58
Glycine	0.74
Alanine	0.77
Histidine	0.48
Arginine	1.24
Fisher ratio	2.49

TABLE 3. GLC analysis of fatty acids of the special formula B (as percentage of total fatty acids).

Fatty acids	Formula B
C6	1.2
C8	5.2
C10	3.8
C12	28.4
C14	11.3
C16	9.8
C16:1	0.4
C18:0	3.2
C18:1	13.6
C18:2	–
C18:3	18.4
% Medium chain fatty acids	38.6

The prepared special foods contain food sources that have appreciable levels of branched chain amino acids, high Fisher ratio, source of medium chain triglycerides and antioxidants. 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.

Soybean protein has been reported to contain a 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 [Oomah, 2001]. Casein also has high Fisher ratio (2.08) according to Food Composition Table [1982]. So, both soybean and casein were incorporated in one of special formula (B). In addition, branched chain amino acids were added in formula B. While the other formula (A) contains whey protein that has been cited previously to contain appreciable levels of branched chain amino acids [Mackle *et al*., 1999]. Whey protein has been reported also to be effective in improving liver dysfunction in chronic hepatitis through its antioxidant activity and an effect related to interferon level [Wong *et al*., 1996; Watanabe *et al*., 2000]. Coconut oil rich in medium chain triglyceride was added [Cottrell, 1991] in both formulas.

Antioxidant phytochemicals especially phenolics present in grains, fruits and vegetables rendered them antioxidant activity [Zamora *et al.*, 1999; Lee *et al*., 2000; Yu *et al*., 2002]. So, in the present study fruits, grains and vegetables that contain antioxidant component were incorporated in the special formulas. Raisin and dates that were present in the formulas has been reported to posses both anti-inflammatory and antioxidant activity [Mohamed & Al-Okbi, 2004; Abdel Fatah *et al*., 2009]. Honey is considered also as a source of antioxidants [Chen *et al*., 2000] so, it is used as sweetener. It has been reported previously that synergistic antioxidant activity has been reported on combination of different antioxidants with each others [Paiva & Russell, 1999], so different sources of antioxidant have been added in the formulas in the current study.

Cynara scolymus and *Nigella sativa* have been used as ingredients of one of the special formulas (A) since they have been reported previously to have beneficial effect towards cirrhotic liver patients [Speroni *et al*., 2003; Al-Ghamdi, 2003]. *Cynara scolymus* has been shown to have antioxidant activity due to the presence of vitamin C, hydroxycinnamic acids, and flavones [Antonio *et al*., 2003].

Patients with liver cirrhosis need establishment of positive nitrogen balance [Molleston, 1996]. So, the present special foods were formulated with appreciable percentage of protein 19.1% and 17.6 for formulas A and B, respectively. The protein used in the formulated foods is of high Fisher ratio in order not to develop encephalopathy in liver cirrhotic patients. Patient with chronic liver disease have increased energy expenditure [McCullough & Tavill, 1991] and most energy utilized in cirrhotic is derived from fat [Schnuwiss *et al*., 1993]. So, appreciable amount of coconut oil as medium chain triglycerides oil was added to elevate the calorific contents of the formulas. In liver disease hepatocytes may have diminished ability to synthesis or secrete bile salts so malabsorption of fat and fat soluble vitamins results [Molleston, 1996]. Medium chain triglycerides are absorbed without the need of bile and facilitate the absorption of long chain fatty acids and oil soluble vitamins [Kaufman *et al*., 1987; Novy & Schwarz, 1997]. So it can improve utilization of fat and fat-soluble vitamins in liver cirrhotic patients. Corn oil as a source of essential fatty acids has been added in formula B expecting that its contents of fatty acids may be absorbed with the aid of the medium chain fatty acids.

Sensory evaluation of the formulas

The results of sensory evaluation of the two formulas are shown in Table 4. The scores of different sensory parameters showed acceptability of both formulas, however overall score of formula A was significantly higher than that of formula B. In the present study, it was decided to carry out the sensory evaluation *via* liver cirrhotic patients this is because those patients usually have anorexia due to taste/appetite changes during dysfunction. So, testing acceptability of special food by cirrhotic patients is better than normal healthy subjects.

Nutritional status and biochemical parameters of liver cirrhotic patients

Assessment of nutritional status was carried out through anthropometric parameters (Tables 5 and 6) and food intake (Table 7). Body mass index (Table 6) revealed that 15% of patients were normal, 46% were overweight and 39% moderately obese. Mid arm circumference results (Table 6) showed that 15% of liver cirrhotic patients were normal and 85% were over normal. Results of muscle circumference (Table 6) showed general reduction of protein compartment. Severe reduction of muscle circumference has been noticed in 54% of cases, moderate reduction in 38% and mild reduction in 8%. This reflects the reduction in body protein compartment in liver cirrhotic patients. Previously Maio *et al*. [2004] suggested

TABLE 4. Parameters of sensory evaluation of the special formulas.

Parameters	Formula A	Formula B
Appearance	7.4±0.3	6.2**±0.2
Crumb colour	7.9±0.3	6.0***±0.4
Texture	8.1±0.3	6.0***±0.4
Taste	7.7±0.2	6.7*±0.3
Overall acceptability	8.1±0.3	6.3***±0.2

Values significantly differ from formula A: *p<0.010, **p<0.005, ***p<0.001.

TABLE 5. Different anthropometric parameters of liver cirrhotic patients (n = 13).

Parameters	Mean ± SD
Age (years)	49.6±9.8
Weight (kg)	82.2±13.1
Height (m)	1.7±0.1
Body mass index (kg/m^2)	28.5±4.5
Mid arm circumference (cm)	28.4±2.6
Triceps skin fold thickness (mm)	14.9±3.1
Mid arm muscle circumference (cm)	18.7±1.0

TABLE 6. Nutritional status of liver cirrhotic patients.

Nutritional status	Mean±SD	No. of cases	Percent*
According to Body mass index (kg/m^2)			
Normal	22.0±2.7	2	15
Overweight	26.9±1.4	6	46
Obese:			
Moderate obesity	33.0±2.4	5	39
According to mid arm circumference (cm)			
Normal level	25.0±0.0	2	15
Over normal	27.7±1.2	11	85
According to muscle circumference (cm)			
Severe reduction	17.9±0.6	7	54
Moderate reduction	19.5±0.3	5	38
Mild reduction	20.2	1	8
According to triceps skin fold (mm)			
Severe malnutrition	8.0	1	8
Normal	13.0 ± 0.0	5	38
Over normal	14.3±1.3	7	54

*The percentage was approximated to the nearest whole number.

that protein-energy malnutrition (PEM) present in cirrhotic patients is predominantly in their protein compartment and worsened with the severity of hepatocellular insufficiency. They reported that upper arm circumference can be used as a sensitive marker of the presence and severity of PEM in cirrhotic patients. It has been cited that hepatic protein synthesis is limited in chronic liver disease including cirrhosis [Marchesini *et al*., 1983]. It was cited that, liver cirrhosis was characterised by significant reduction in body cell mass and body fat and by a redistribution of body water. Significant losses occurred even in patients with mild disease. There was a more pronounced loss of fat in the initial stage, followed by accelerated loss of body cell mass in the advanced stages of liver cirrhosis [Figueiredo *et al*., 2005].

Triceps skin fold (Table 6) showed 54% of cases were overweight while 38% were normal and 8% of cases showed severe malnutrition. As a matter of fact, careful anthropometric studies showed that weight and BMI underestimate the degree of malnutrition in chronic liver disease when compared to triceps skin fold thickness, this probably occurs because organomegaly and ascites artificially inflate the weight [Molleston, 1997]. So, according to the previous author, in the present study TSF may reflect the actual nutritional status of patients. However the overall picture of anthropometric parameters showed protein malnutrition in liver cirrhotic patients.

Mean dietary intake of patients at the beginning of the study (Table 7) revealed that all liver cirrhosis patients were hypocaloric. All estimated nutrients were lower than RDA except for protein (112% of RDA). Despite the high intake of protein, there was reduction of body protein compartment as noticed from the anthropometric parameters in the present study emphasizing on the impaired protein metabolism in liver cirrhotic patients. Deficient intake of vitamin E (27% of RDA), vitamin C (65% of RDA), zinc (55% of RDA), Cu (49% of RDA) and iron (81% of RDA) was noticed. The reduction in intake

TABLE 7. Mean daily dietary intake of different nutrients at the beginning of the study.

Parameters	Nutrient intake Mean±SE	%RDA
Energy (cal.)	1964.0±45.7	68
Carbohydrate (g)	226.2±10.3	-
Protein (g)	70.7±4.2	112
Animal protein	39.6±4.8	-
Fat(g)	63.2±3.5	-
Saturated Fat	30.1±3.1	-
Monounsaturated Fat	19.7±0.9	-
Polyunsaturated Fat	10.6±1.6	-
Vit. E (mg)	2.7±0.3	27
Vit. C (mg)	39.1±9.0	65
Cu (mg)	1.1±0.1	49
Iron (mg)	8.1±0.6	81
Zinc (mg)	8.3±0.3	55

of antioxidant vitamins E and C and the trace elements; zinc and Cu may elevate oxidative stress in those patients that may lead to deterioration of disease state.

Mean dietary intake of patient after two months of the study (Table 8) revealed that all liver cirrhosis patients though increased their caloric intake however they were still hypo-caloric. Protein intake was increased to 136 and 120% from RDA in patients taking Formula A and B, respectively. Patients taking formula (A) showed increased in animal protein intake from 56% (of total protein intake) at the beginning of the study to 65% at the end of the study. It was noticed that patients followed the dietary advice through increasing the intake of skimmed milk and milk products (of high Fisher ratio) at the expense of meat. In addition, the intake of protein supplemented from both special formulas was of high Fisher ratio. Thereby increased protein intake of such quality did not represent any load on the liver. Patients in both groups showed increase in minerals and vitamins intake as a result of dietary advice. As a matter of fact mean dietary intake was calculated without the nutrient intake supplemented from the special formulas.

Table 9 showed the different biochemical parameters of liver cirrhotic patients at the beginning of the study (before dietary supplement) in comparison to healthy subjects. As expected, liver dysfunction was noticed in liver cirrhotic patients reflected by the significant increase in the activities of AST, ALT and ALP and total and direct bilirubin. As a matter of fact, the severity of liver synthetic dysfunction is estimated by measuring bilirubin [Molleston, 1997]. The significant decrease of plasma albumin and total protein in cirrhotic patients seen in Table 9, clarified liver synthetic dysfunction of protein. Plasma glucose showed only non-significant increase compared to control.

Ammonia was significantly high in liver cirrhotic patients compared to normal. Hyperammonemia has been reported in chronic liver disease [Haussinger, 1990] which resulted from the reduced capacity of the sick liver to convert ammonia into urea. High oxidative stress in chronic liver disease dem-

TABLE 8. Mean daily dietary intake of different nutrients at the end of the study.

Parameters	Nutrient intake (Mean±SE)				RDA
	Group given formula A	% RDA	Group given formula B	% RDA	
Energy (cal.)	1992.0±22.6	69	2005.0±13.2	69	2900
Carbohydrate (g)	252.8±9.3	-	281.3±8.8	-	-
Protein (g)	85.9±9.1	136	75.6±5.4	120	63
Animal protein	55.9±9.9	-	42.3±6.2	-	-
Fat (g)	68.6±5.2	-	61.4±2.3	-	-
Saturated Fat	29.6±2.4	-	24.9±2.7	-	-
Monounsaturated Fat	21.9±1.8	-	19.0±0.7	-	-
Polyunsaturated Fat	10.8±2.0	-	11.2±2.3	-	-
Vit. E (mg)	3.7±0.8	37	2.9±0.3	29	10
Vit. C (mg)	51.4±12.5	86	41.3±2.0	69	60
Cu (mg)	1.1±0.1	49	1.2±0.1	53	1.5-3
Iron (mg)	8.3±0.4	83	8.6±0.7	86	10
Zinc (mg)	8.8±0.3	59	8.7±0.5	58	15

TABLE 9. Different biochemical parameters of healthy subjects and liver cirrhotic patients before dietary supplementation.

Plasma parameters	Healthy subjects Mean±SE	Liver cirrhotic patients Mean±SE
Glucose (mg/dL)	76.5±1.8	96.1±7.5
% Change		26
AST (U/L)	7.4±0.2	41.4**±1.8
% Change		459
ALT (U/L)	6.6±0.6	17.3**±1.3
% Change		162
ALP(IU/L)	12.1±0.5	24.9**±1.2
% Change		106
T. Bilirubin (mg/dL)	0.4±0.02	0.7**±0.1
% Change		75
D. Bilirubin (mg/dL)	0.1± 0.0	0.4**±0.1
% Change		300
T. Protein (g/dL)	7.5±0.1	6.7**±0.1
% Change		-11
Albumin (g/dL)	3.9±0.1	3.4**±0.05
% Change		-13
Golbulin (g/dL)	3.6±0.1	3.5±0.1
% Change		-3
A/G ratio	1.1±0.1	0.98*±0.04
% Change		-11
Ammonia (Umol/L)	25.4±1.3	252.6**±6.9
% Change		894
Nitric oxide (umol/L)	7.6±0.21	18.6**±0.5
% Change		145
MDA (nmol/L)	4.4±0.21	14.6**±0.3
% Change		232

Values statistically significant when patients compared with healthy subjects: *p<0.01, **p<0.001.

TABLE 10. Different biochemical parameters of liver cirrhotic patients before and after 2-month dietary intervention with the two formulas.

Plasma parameters	Group given formula A		Group given formula B	
	Before Mean±SE	After Mean±SE	Before Mean±SE	After Mean±SE
Glucose (mg/dL)	103.6±13.2	97.6±10.5	87.3±4.7	85.2±4.6
% Change		-6		-2
AST (U/L)	41.7±2.5	21.6**±1.3	41±2.9	29.5±2.2
% Change		-48		-28
ALT (U/L)	14.7±0.9	12.4±0.8	20.3±2.2	17±1.5
% Change		-16		-16
ALP(IU/L)	24.1±1.9	19.1±1.9	25.9±1.3	21.5±0.8
% Change		-21		-17
T. Bilirubin (mg/dL)	0.8±0.2	0.6±0.1	0.6±0.1	0.5±0.1
% Change		-25		-17
D. Bilirubin (mg/dL)	0.4±0.1	0.3±0.1	0.33±0.1	0.29±0.1
% Change		-25		-12
T. Protein (g/dL)	6.9±0.1	7.3**±0.1	7.1±0.1	7.4***±0.1
% Change		6		4
Albumin (g/dL)	3.4±0.1	3.7****±0.04	3.4±0.1	3.7***±0.04
% Change		9		9
Golbulin (g/dL)	3.5±0.2	3.6±0.1	3.6±0.1	3.8±0.1
% Change		3		6
A/G ratio	1.0±0.1	1.03±0.03	0.9±0.03	1.0 ±0.03
% Change		3		11
Ammonia (Umol/L)	268.9±5.2	128.2****±3.0	233.7±8.7	171.4±7.3
% Change		-52		-27
Nitric oxide (umol/L)	19.1±0.9	15.4*±0.7	17.9±0.4	15.8**± 0.6
% Change		-19		-12
MDA (nmol/L)	14.6±0.5	11.6***±0.5	14.6±0.2	12.1****±0.3
% Change		-21		-17

Values statistically significant when data after intervention were compared with that before: *p<0.025, **p<0.01, ***p<0.005, ****p<0.001.

onstrated previously by Sumida *et al*. [2003] was emphasized in the present study by the significant elevation of MDA. Nitric oxide which is considered as bio marker of inflammation and oxidative stress was significantly high in liver cirrhotic patients in the current study. 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 [Cabre & Gassull, 1999]. This oxidation might be contributed by elevation of oxidative stress in those patients.

Table 10 showed biochemical parameters of patients before and after dietary intervention and nutritional advice. It can be noticed that virtually all parameters reflecting liver dysfunction non-significantly improved after dietary intervention of either special formulas. The only significant improvement was the significant decrease of AST on supplementation of diet A.

A significant increase in both plasma albumin and total protein was noticed in patients after both dietary intervention which may reflect some improvements in liver synthetic function and nutritional status. Plasma ammonia level showed pronounced reduction after both dietary supplementations which was significant in patients given formula A. Nitric oxide and MDA were significantly reduced in both dietary interventions with different degrees, resulting in reduction in oxidative stress and inflammation.

CONCLUSIONS

In conclusion, both dietary interventions in addition of dietary advice in the present study have beneficial effects towards liver cirrhotic patients concerning reduction of oxidative stress and inflammation. Formula A was superior in reducing plasma AST activity and ammonia level.

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