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EFFECT OF LIVERCARE ON PROTEIN ANABOLISM: A PROSPECTIVE, RANDOMIZED, TRIPLE-BLIND, PLACEBO-CONTROLLED CLINICAL STUDY

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ABSTRACT

Background: The term anabolism is defined as a set of metabolic processes that makes use of the energy released by catabolism to synthesize complex molecules in a constructive sequence for the body. Known to play a significant role in anabolic processes, liver is an important metabolic centre in the body. Various drugs are identified to have a stimulating effect and a protective influence on the liver parenchyma, but of late safer herbal supplements are preferred over the commonly used testosterone analogues for anabolic benefits. One such herbal supplement, LiverCare promotes anabolism of proteins and improves liver function, protein synthesis and metabolism. **Objective:** The primary objective of this clinical study was to evaluate the protein anabolic activity of LiverCare in healthy adults while the secondary objectives were to evaluate the safety profile of the formulation and compliance to the study. **Design:** The healthy individuals included in this triple-blind, placebo-controlled study were administered LiverCare (investigational product) or placebo at a dose of one capsule twice daily for 60 days. The effect of the investigational product was evaluated by estimating nitrogen retention using total nitrogen excretion (urinary, fecal and total), serum testosterone and serum cortisol levels. **Results and Conclusion:** Results of the study showed that LiverCare decreased the fecal and urinary nitrogen excretion, thereby maintaining a positive nitrogen balance. Excretion of urinary urea was also reduced. No adverse effects were observed and the medication was well-tolerated.

KEYWORDS: LiverCare, protein anabolism, total nitrogen excretion, anthropometric data.

INTRODUCTION

Nutrition plays an essential role in Ayurveda, a traditional system of medicine native to India. Ayurveda considers "Ahara" (diet) and "Anna" (food) to be the fundamental ingredients for a good and healthy life and overall wellness and believes that healthy and wholesome food nourishes the mind, body and soul. Ayurveda asserts that though digestive capacity may differ from individual to individual, the quality and appropriate quantity of food are necessary for a healthy life.[1] Food taken in proper quantity provides strength, vigor and good complexion, and nurtures the health of the tissues.[2,3]

In contrast to the Western dietary understanding and the U.S. guide to diet, Ayurveda states that a diet can be either vegetarian (plant-based) or non-vegetarian (animal-based) and the portion size should be customized for each individual according to one's own needs, body constitution (*dosha*) and *agnibal* (digestive power). Moreover, the quality and property of food, such as heavy, light or oily, are also taken into consideration in Ayurvedic diet.[1]

When food is taken into the body, metabolic activities consisting of catabolism (degradation) and anabolism (biosynthesis) begin. While catabolism is the set of metabolic pathways that breaks down molecules into smaller units, anabolism makes use of the energy released by catabolism to synthesize complex molecules in a constructive sequence for the body. Production of precursors such as amino acids, monosaccharides, isoprenoids and nucleotides; activation of precursors into reactive forms using energy from ATP; and assembly of these precursors into complex molecules such as proteins, polysaccharides, lipids and nucleic acids contribute to anabolism. Several factors such as caloric intake, digestion, absorption, utilization and endocrine functioning influence anabolism.

Several recent studies have shown that the maximum stimulation of muscle protein fractional synthetic rate occurs with the intake of 20 to 30 gm of protein, leading to the concept of a maximal anabolic response to protein intake with a meal. Higher protein intake when protein synthesis is maximized is characterized by suppressed protein breakdown, which leads to greater anabolic response. This explains the reason for a linear relationship between amino acid availability and net gain, without any apparent plateau of effect at higher levels of availability when net protein synthesis is measured.[4]

Known to play a significant role in anabolic processes, liver is an important metabolic centre in the body. Various drugs are identified to have a stimulating effect and a protective influence on the hepatic parenchyma. The most commonly used drugs for anabolic benefits are testosterone analogues. The ergogenic effects associated with these drugs include increase in lean body mass, increase in cross-sectional area of the muscle, decrease in body fat percentage, increase in muscle strength and power, increase in pain tolerance and behavior modification.[5]

However, several systemic adverse effects such as risks of liver tumors and testicular atrophy, changes in lipid profile and psychological effects have been observed with the use of anabolic steroids, some of which are even irreversible.[6] Moreover, anabolic steroids are banned in several countries due to safety concerns.

As an alternative to anabolic steroids, several herbal medicines are commercially available that are potent anabolic actives, which are known to improve appetite and rate of nutrient absorption. However, these supplements are often promoted without any conclusive research demonstrating their efficacy.[7] A recent review of 250 commercially advertised supplements found that only six of these had been examined in randomized, placebo-controlled studies greater than 3 weeks in duration.[8]

One of the herbal supplements, LiverCare is said to promote anabolism of proteins and improve liver function, protein synthesis and metabolism. The aim of this study was to study the efficacy and safety of the role of LiverCare on protein anabolism.

MATERIALS AND METHODS

Inclusion Criteria

Healthy male and female volunteers aged between 18 and 40 years who were willing to participate in the clinical study and follow the study procedures were included in the study.

Exclusion Criteria

Subjects with severe metabolic disorders; known history or present condition of allergic response to similar pharmaceutical products, its components or ingredients in the test products; pre-existing systemic disease necessitating long-term medications; and genetic and endocrinal disorders were excluded from the study. Subjects who had participated in a similar

clinical investigation in the past four weeks or used a similar product in the past four weeks as well as pregnant and lactating women were excluded from the study.

Study Design

Seventy subjects were included in the study and randomized equally into two groups namely, LiverCare capsules group and placebo group in a triple-blind design where neither the investigator nor the subject or sponsor (except for the pharmacist handling the drugs) was aware of the assigned formulation.

Study Procedure

The study protocol, CRFs, regulatory clearance documents, product-related information and informed consent form were submitted to and approved by the Institutional Ethics Committee. Eligible subjects who were willing to participate in the study were included in the study after they willingly signed the informed consent. During the initial visit, a detailed medical history was obtained from all subjects with a special emphasis on the medical history of their family members. All subjects underwent a thorough systemic examination and hematological and biochemical tests to ensure they were in good health. The subjects were advised to consume LiverCare or a similar looking placebo orally at a dose of one capsule twice daily before meals for a period of 60 days and not to take any vitamins or general tonics during the course of the trial. Subjects were also advised to restrict their calorie intake between 2500 and 2800 cal/day (including regular quantity of protein).

All adverse events, either reported or observed by subjects, were recorded in the CRF with information about severity, onset, duration and action taken regarding the study drug. Relation of adverse events to the study medication was predefined as "*Unrelated*" (does not follow a reasonable temporal sequence from the time of administration of the drug), "*Possible*" (follows a known response pattern to the suspected drug, but could have been produced by the patient's clinical state or other modes of therapy administered to the patient), and "*Probable*" (follows a known response pattern to the suspected drug that could not be reasonably explained by the known characteristics of the patient's clinical state).

Subjects were allowed to voluntarily withdraw from the study, if they experienced serious discomfort during the study or sustained serious clinical events requiring specific treatment. For subjects withdrawing from the study, efforts were made to ascertain the reason for dropout.

Study Follow-up and Monitoring

Response to treatment was evaluated by nitrogen retention tests, namely total urinary nitrogen content, total fecal nitrogen content and urinary urea at entry and on days 9, 18, 28 and 60. Serum testosterone and serum cortisol levels were evaluated at entry and on days 28 and 60.

Hematological and clinical biochemical parameters were analyzed to establish the safety profile of the formulation.

Primary and Secondary End Points

The primary objective of the study was to evaluate the protein anabolic activity of LiverCare in healthy adults by maintaining a positive nitrogen balance, which was measured by reduced fecal and urinary nitrogen excretion and urinary urea and improvement in hormones responsible for protein anabolism such as serum testosterone and cortisol.

The secondary objectives of the study were to evaluate the safety profile of the formulation, which was assessed by adverse events and laboratory investigations (including hematology as well as clinical chemistry parameters (LFT and RFT) and compliance to the study.

STATISTICAL ANALYSIS

Statistical analysis was carried out according to the intention-to-treat principles. Values are expressed as Mean ± SD. Unpaired t-test for between-the-group analysis using two-tailed *p* value of <0.05 was considered significant. Statistical analysis was performed using GraphPad Prism, Version 4.03 for Windows, Graph Pad Software, San Diego, California, United States.

RESULTS

The enrolled subjects were randomized into two groups (LiverCare and placebo) of 35 individuals each. The mean age of the subjects in the LiverCare group was 22.82 ± 5.11, while in the placebo group it was 22.74 ± 5.18. Sex ratio was 30:5 and 26:9 (M:F) for LiverCare and placebo groups, respectively. (**Table 1**).

Baseline values of total urinary nitrogen content, fecal nitrogen content and urinary urea are mentioned in **Table 2**. Total urinary nitrogen content in LiverCare and placebo groups were 1.55 ± 0.51 and 1.20 ± 0.44, respectively with no significance between the groups at baseline whereas the total fecal nitrogen content in LiverCare and placebo groups were 0.98 ± 0.15 and 0.98 ± 0.12, respectively with no significance between the groups. Urinary urea values in

the LiverCare and placebo groups were 3.31 ± 1.09 and 2.60 ± 0.95, respectively. Statistical analysis was done using unpaired t-test for between-the-group analysis using two-tailed *p* value.

Effect of the treatment on fecal nitrogen content, urinary nitrogen content and urinary urea (values presented as the difference from baseline) is mentioned in **Table 3**. Statistical analysis was done using unpaired t-test for between-the-group analysis using two-tailed *p* value. The fall in the total fecal nitrogen content as compared to baseline (**Figure 1**) on Day 9 was -0.11 ± 0.14 and 0.01 ± 0.11 in LiverCare and placebo groups, respectively with a significance of $p<0.0001$ between the groups, while on Day 60, the fall in total fecal nitrogen content as compared to baseline was -0.08 ± 0.18 and 0.06 ± 0.22 in LiverCare and placebo groups with a significance of $p<0.0047$.

The fall in urinary nitrogen content (**Figure 2**) on Day 9 as compared to baseline was -0.01 ± 0.71 in LiverCare group and 0.03 ± 0.63 in placebo group with no significance. On Day 60, the urinary nitrogen content as compared to baseline had decreased to -0.32 ± 0.60 and 0.06 ± 0.75, respectively in LiverCare and placebo groups with a significance of $p<0.0225$.

Similarly, the fall in the urinary urea level (**Figure 3**) on Day 9 was -0.02 ± 1.51 and 0.14 ± 1.34 in LiverCare and placebo groups, respectively with no significance between the groups, while on Day 60 it was -0.57 ± 1.46 and 0.22 ± 1.59 in LiverCare and placebo groups, respectively with a significance of $p<0.0347$.

The effect of LiverCare on total nitrogen excretion is tabulated in **Table 4**. Total nitrogen excretion was 5.83±1.62 at baseline and 6.22±1.78 in LiverCare group at the end of 60 days, while the placebo group was 4.78±1.37 at baseline and 5.79±1.58 at the end of the study with a significance of $p<0.0063$. Total nitrogen loss was comparatively less in the LiverCare group as compared to the placebo group, which signifies the anabolic effect of LiverCare.

Values of total nitrogen excretion after normalization are provided in **Table 5**. The total nitrogen excretion after normalization in LiverCare group on Day 9 as compared to baseline was 0.24±1.64, which further decreased to -0.72±1.81 on Day 60 with a significance of $p<0.0343$. In the placebo group, the decrease was -0.31±1.65 on Day 60 as compared to -0.24±1.40 on Day 9. These values clearly indicate a decline in nitrogen excretion between Day 9 and Day 60, demonstrating the anabolic effect of LiverCare.

The effect of the therapy on endocrinal parameters is shown in **Table 6**. Serum testosterone levels on Day 0 were 402.80 ± 206.50 and 335.00 ± 212.20, respectively in LiverCare and placebo groups with no significance. On Day 60, the testosterone levels showed an increasing trend without any significance, having values of 560.80 ± 265.50 and 498.70 ± 300.50, respectively for LiverCare and placebo groups. Though there is a trend towards an increase in both the groups, no significance was observed. Serum cortisol levels on Day 0 were 14.63 ± 5.70 and 15.34 ± 6.16, respectively in LiverCare and placebo groups with no significance. On Day 60, cortisol levels were at 17.78 ± 4.78 and 17.33 ± 5.41 in placebo group with no significance. Statistical analysis was done using unpaired t-test for between-the-group analysis using two-tailed *p* value.

The effect of LiverCare on weight gain is tabulated in **Table 7**. Weight improved from 75.72± 9.34 to 77.48±9.49 in LiverCare group at the end of 60 days, while the placebo group showed improvement from 71.81±10.07 to 72.13±10.39 at the end of the study. Between the group analysis of LiverCare with Placebo showed a significance of $p<0.0278$. Within the group analysis of LiverCare on Day 60 as compared to Day 0 values showed a significance of $p<0.0001$. Within the group analysis of Placebo on Day 60 as compared to Day 0 values showed a significance of $p<0.0013$.

The effects of the treatment on hematological and biochemical parameters are shown in **Tables 8 and 9**. All values are within normal range, demonstrating the safety profile of the product.

Table 1: Demographic data of subjects on entry (n=70)

Parameters	LiverCare	Placebo
Number of subjects	35	35
Age in years (mean ± SD)	22.82 ± 5.11	22.74 ± 5.18
Sex		
Male	30	26
Female	5	9

Table 2: Baseline values of total urinary nitrogen content, fecal nitrogen content and urinary urea

Parameters	LiverCare	Placebo	Significance
Total nitrogen content – urinary (g/dl)	1.55 ± 0.51	1.20 ± 0.44	NS
Total nitrogen content – fecal (g%)	0.98 ± 0.15	0.98 ± 0.12	NS
Urinary urea (g/dl)	3.31 ± 1.09	2.60 ± 0.95	NS
Statistical analysis: Unpaired t-test for between-the-group analysis using two-tailed *p* value. NS: Not significant.			

Table 3: Effect of the therapy on urinary nitrogen content, fecal nitrogen content and urinary urea (values presented as the difference from baseline)

Parameter	Day	LiverCare	Placebo	Significance
Total nitrogen content – urinary (g/dl)	Day 9	-0.01 ± 0.71	0.03 ± 0.63	NS
	Day 18	-0.47 ± 0.52	0.05 ± 0.70	p<0.0006
	Day 28	-0.13 ± 0.69	0.29 ± 0.41	p<0.0032
	Day 60	-0.32 ± 0.60	0.06 ± 0.75	p<0.0225
Total nitrogen content – fecal (g%)	Day 9	-0.11 ± 0.14	0.01 ± 0.11	p<0.0001
	Day 18	-0.06 ± 0.17	0.05 ± 0.22	p<0.0235
	Day 28	-0.08 ± 0.16	0.05 ± 0.18	p<0.0027
	Day 60	-0.08 ± 0.18	0.06 ± 0.22	p<0.0047
Urinary urea (g/dl)	Day 9	-0.02 ± 1.51	0.14 ± 1.34	NS
	Day 18	-0.47 ± 0.99	0.04 ± 1.19	NS
	Day 28	-0.44 ± 1.25	0.41 ± 1.00	p<0.0028
	Day 60	-0.57 ± 1.46	0.22 ± 1.59	p<0.0347

Statistical analysis: Unpaired t-test for between-the-group analysis using two-tailed *p* value.
NS: Not significant.

Table 4: Effect of LiverCare on total nitrogen excretion (g%)

Treatment	Treatment days (mean± SD)		Significance
	Day 0	Day 60	
LiverCare	5.83 ± 1.62	6.22 ± 1.78	**NS**
Placebo	4.78 ± 1.37	5.79 ± 1.58	**p<0.0063**

Total nitrogen excretion (fecal + urine + urinary urea nitrogen)
Statistical analysis: paired t-test for within-the-group analysis using two-tailed *p* value.
NS: Not significant.

Table 5: Total nitrogen excretion after normalization (g%)

Treatment	Treatment days (mean ± SD)		Significance
	Day 9	Day 60	
LiverCare	0.24 ± 1.64	-0.72 ± 1.81	p<0.0343
Placebo	-0.24 ± 1.40	-0.31 ± 1.65	NS

Total nitrogen excretion (fecal + urine + urinary urea nitrogen)
Statistical analysis: paired t-test for within-the-group analysis using two-tailed *p* value.
NS: Not significant.
Normalization indicates the difference between the baseline value and Day 9/Day 60 value.

Table 6: Effect of drug therapy on endocrinal parameters.				
Parameter	**Day**	**LiverCare**	**Placebo**	**Significance**
Serum testosterone (ng/dl)	Day 0	402.80 ± 206.50	335.00 ± 212.20	NS
	Day 9	410.20 ± 192.10	335.70 ± 209.60	NS
	Day 60	560.80 ± 265.50	498.70 ± 300.50	NS
Serum cortisol (µg/dl)	Day 0	14.63 ± 5.70	15.34 ± 6.16	NS
	Day 9	18.59 ± 6.76	17.25 ± 6.38	NS
	Day 60	17.78 ± 4.78	17.33 ± 5.41	NS
Statistical analysis: Unpaired t-test for between-the-group analysis using two-tailed *p* value. NS: Not significant.				

Table 7: Effect of treatment on Weight			
Weight	**LiverCare**	**Placebo**	**Significance**
Day 0	75.72± 9.34	71.81±10.07	NS
Day 60	77.48±9.49[a,b]	72.13±10.39[c]	[a]p<0.0278 [b]p<0.0001 [c]p<0.0013
Statistical analysis: Unpaired t test NS:Non Significant **Significance** a-Between the group analysis-LiverCare as compared to Placebo b,c-Within the group analysis –Day 60 values compared to Day 0			

Table 8: Effect of drug therapy on hematological parameters				
Parameter	**Day**	**LiverCare**	**Placebo**	**Significance**
Hemoglobin	Day 0	13.96 ± 1.047	13.93 ± 1.076	NS
	Day 9	14.55 ± 1.16	14.57 ± 1.226	NS
	Day 60	14.74 ± 1.414	15.81 ± 6.431	NS
TLC count /cu.mm.	Day 0	6300 ± 1448	6257 ± 1084	NS
	Day 9	6154 ± 1284	6449 ± 1199	NS
	Day 60	6191 ± 1581	6057 ± 1516	NS
Granulocytes %	Day 0	55.76 ± 5.26	59.2 ± 6.37	NS
	Day 9	55.98 ± 6.61	61.39 ± 6.22	p<0.0008
	Day 60	57.39 ± 6.56	60.06± 6.25	NS
Mid %	Day 0	9.543 ± 1.68	9.289 ± 1.64	NS
	Day 9	9.523 ± 1.44	8.691± 1.73	p<0.0323
	Day 60	8.949 ± 1.68	8.657±1.81	NS
Lymphocytes %	Day 0	34.7 ± 4.75	31.51 ± 6.03	NS
	Day 9	34.5 ± 5.92	29.91 ± 5.77	p<0.0016
	Day 60	33.67 ± 6.16	31.28 ± 5.99	NS
ESR (mm/h)	Day 0	9.086 ± 3.17	7.486 ± 2.49	NS
	Day 9	9.429 ± 4.32	8.286 ± 2.05	NS
	Day 60	9.4 ± 3.05	9.571 ± 3.70	NS
Statistical analysis: Unpaired t-test for between-the-group analysis using two-tailed *p* value. NS: Not significant.				

Table 9: Effect of drug therapy on clinical chemistry parameters.

Parameter	Day	LiverCare	Placebo	Significance
TGO - AST (U/L) (Aspartate transaminase)	Day 0	26.58± 13.26	23.47± 8.88	NS
	Day 9	25.54± 11.88	22.83± 8.33	NS
	Day 60	19.67± 4.78	19.46± 6.25	NS
TGP - ALT (U/L) (Alanine transaminase	Day 0	29.70± 18.18	26.30± 13.10	NS
	Day 9	27.02± 14.36	24.28± 12.28	NS
	Day 60	23.78± 11.97	22.11± 10.30	NS
ALP (U/L) (Alkaline phosphatase)	Day 0	165.00±61.17	159.30± 61.55	NS
	Day 9	164.40± 45.57	166.40± 68.15	NS
	Day 60	181.10± 54.93	183.70± 81.98	NS
Total bilirubin (mg/dl)	Day 0	0.82± 0.37	0.67± 0.30	NS
	Day 9	0.77± 0.35	0.58± 0.34	p<0.0256
	Day 60	0.75± 0.38	0.58± 0.31	p<0.0397
Serum creatinine (mg/dl)	Day 0	0.80± 0.09	0.78± 0.08	NS
	Day 9	0.82± 0.07	0.84± 0.11	NS
	Day 60	0.85± 0.09	0.84± 0.10	NS
Serum urea (mg/dl)	Day 0	34.00 ± 6.34	31.21 ± 7.32	NS
	Day 9	36.11 ± 8.42	31.56 ± 6.77	p<0.0152
	Day 60	34.81 ± 6.46	32.13± 7.51	NS
Serum lipid (mg/dl)	Day 0	570.70± 130.90	532.10± 101.80	NS
	Day 9	551.90 ±119.10	522.80 ±105.50	NS
	Day 60	587.50 ± 120.20	577.70 ±122.00	NS
Serum total cholesterol (mg/dl)	Day 0	172.70 ± 36.79	168.00 ± 36.74	NS
	Day 9	164.30 ± 34.21	159.60 ± 35.51	NS
	Day 60	185.10 ± 35.19	179.50 ± 41.12	NS
HDL cholesterol (mg/dl)	Day 0	66.67± 3.94	68.83 ± 4.72	NS
	Day 9	68.94 ± 4.41	68.21 ± 4.07	NS
	Day 60	67.50± 7.54	68.95± 4.76	NS
LDL cholesterol (mg/dl)	Day 0	87.60 ± 33.68	86.31 ± 35.90	NS
	Day 9	79.77 ± 28.39	76.71 ± 34.41	NS
	Day 60	100.70 ± 31.17	94.63 ± 41.42	NS
Serum triglyceride (mg/dl)	Day 0	92.02 ± 68.92	64.1±32.55	p<0.0337
	Day 9	86.93 ± 66.73	73.68 ± 44.05	NS
	Day 60	80.87 ± 55.82	82.42 ± 49.06	NS

Statistical analysis: Unpaired t-test for between-the-group analysis using two-tailed *p* value.
NS: Not significant.

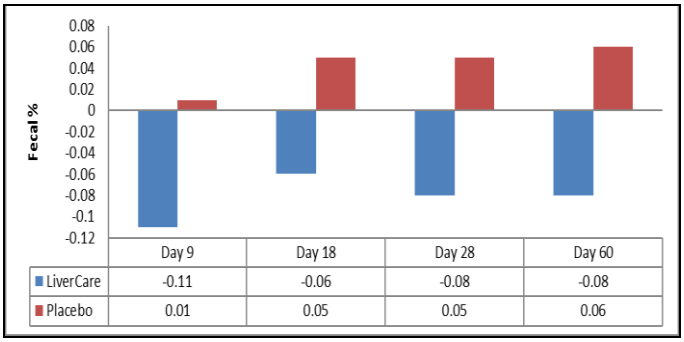

Figure 1: Total nitrogen content – fecal (g%).

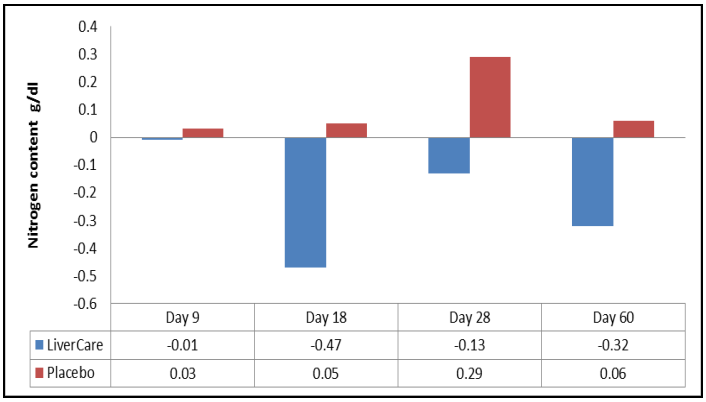

Figure 2: Total nitrogen content – urinary (g/dl).

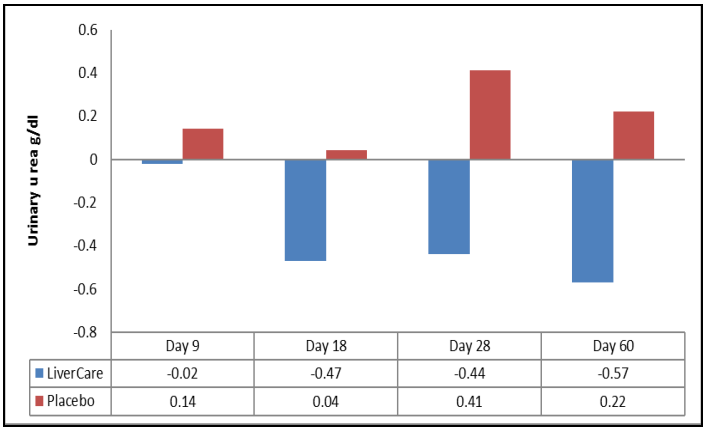

Figure 3: Total nitrogen urea – urinary urea.

DISCUSSION

Liver plays a significant role in intermediary metabolism and its functional integrity is essential for the supply and transport of nutrients such as carbohydrates, fats and proteins. Liver regulates the synthesis, storage and breakdown of glycogen and hepatocytes, the predominant cell type in liver, express enzymes that enable them to synthesize glucose from various precursors such as amino acids, pyruvate and lactate through the process of gluconeogenesis.[9]

Several factors may disrupt this metabolic cycle such as in cases of liver failure, resulting in numerous nutritional problems. Malnutrition in liver failure may occur due to several factors such as inadequate dietary intake of nutrients, reduced protein synthesis and malabsorption, increased protein loss, disturbances in substrate utilization, state of hypermetabolism and increased energy-protein expenditure and requirements. Because of decreased glycogen stores and gluconeogenesis[10], energy metabolism may shift from carbohydrate to fat

oxidation[11] while also developing resistance to insulin. Consequently, impairment of liver function may induce a catabolic state, resulting in lack of essential nutrients.[9]

The net balance between muscle protein synthesis and breakdown distinguishes the catabolic state from the anabolic state. The synthesis of new protein is derived from the intracellular pool of essential amino acids[12], which is the most important determinant of protein synthesis as the non-essential amino acids are readily available.[13]

The changes in breakdown alone cannot induce a shift from catabolic to anabolic state, since some of the amino acids released from protein breakdown are oxidized or transaminated and therefore not available for re-incorporation into protein. On the other hand, the rate of protein breakdown will always be linked to some extent to the rate of synthesis because of the contribution of amino acids from breakdown to the intracellular pool of amino acids. Thus, when considering the role of amino acid availability in regulating the rate of muscle protein synthesis, it is necessary to take into account not only the amino acids from plasma, but also the amino acids that appear in the intracellular pool as a result of protein breakdown.[4]

The poly-herbal combination in LiverCare is known to promote anabolism of proteins and improve liver function to help protein synthesis and metabolism. Results of several studies have shown that extracts of LiverCare capsule act as appetite stimulants. In addition, LiverCare is also shown to improve absorption and utilization of food, regularize bowel movements, facilitate digestion and assimilation processes and promote weight gain. It also possesses hepatoprotective, antioxidant, antimicrobial and anti-inflammatory properties.

Khanfar *et al*. isolated and identified the active ingredients of *Capparis spinosa*, one of the components of LiverCare, as beta-sitosterylglucoside-6'-octadecanoate and 3-methyl-2-butenyl-beta-glucoside.[14] p-Methoxy benzoic acid isolated from *Capparis spinosa* was found to possess potent hepatoprotective activity against CCl_4, paracetamol (*in vivo*) and thioacetamide and galactosamine (*in vitro*)-induced hepatotoxicity.[15]

Results of studies by Aktay *et al*. and Zafar *et al*. demonstrated the hepatoprotective effect (confirmed by histopathological examination) of *Cichorium intybus*, another component of LiverCare, against CCl_4-induced hepatotoxicity and reported significant prevention of the elevation of malondialdehyde formation (plasma and hepatic) and enzyme levels of aspartate transaminase and alanine transaminase.[16,17]

Other ingredients of LiverCare were also studied for their effect on liver and its function. *Solanum nigrum* was investigated for its hepatoprotective activity against CCl_4-induced hepatic damage by Raju *et al*. and they observed remarkable hepatoprotective activity, confirmed by the evaluation of biochemical parameters such as aspartate transaminase, alanine transaminase, alkaline phosphatase and total bilirubin.[18] Sultana *et al*. demonstrated that *Solanum nigrum* protect DNA against oxidative damage and the results suggest that the observed hepatoprotective effect of *Solanum nigrum* might be due to the ability to suppress oxidative degradation of DNA in the tissue debris.[19]

Upadhyay *et al.* isolated arjunetoside, a triterpene glycoside and oleanolic and arjunic acids as active ingredients from *Terminalia arjuna*.[20] Munasinghe *et al*. reported potent antioxidant activity of *Terminalia arjuna*, which might be due to its effects on lipid peroxidation.[21] Ali *et al*. demonstrated that arjunaphthanoloside from *Terminalia arjuna* inhibits nitric oxide production[22] and terminoside A isolated from *Terminalia arjuna* decreases inducible nitric oxide synthase (iNOS) levels in lipopolysaccharide-stimulated peritoneal macrophages.[23]

Bin-Hafeez *et al*. showed that *Cassia occidentalis* modulates hepatic enzymes and provides hepatoprotection against induced immunosuppression.[24] Candan *et al*. and Bezic *et al*. reported antioxidant and antimicrobial activities of *Achillea millefolium*.[25,26]

CONCLUSION

The results of the current clinical trial demonstrated that LiverCare decreases fecal and urinary nitrogen excretion, thereby maintaining a positive nitrogen balance. Excretion of urinary urea was also reduced after LiverCare administration. No adverse effects were observed and the medication was well-tolerated. The synergistic actions (hepatoprotective, antimicrobial, antioxidant and anti-inflammatory) exhibited by the ingredients of LiverCare have a beneficial effect on protein anabolism in healthy individuals. Thus, LiverCare acts as an anabolic support, increasing the functional capacity of the liver accelerating the cellular metabolic activity and promoting regeneration.

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