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Liverubin (standardized silymarin) in the supplementary management of functional, temporary hepatic damage. A pilot, registry, study

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Aim. **Mild, temporary hepatic failure (MTHF) may be completely asymptomatic or cause minimal signs and symptoms. This common clinical problem is very diffuse and, in case of repeated episodes may cause a chronic impairment in liver function. The aim of this registry was to evaluate the evolution of MTHF in subjects using Liverubin (a new standardized Silymarin preparation) over a 4-week period.**
Methods. **Patients with MTHF were observed in a registry study. In all subjects viral hepatitis markers were negative at inclusion. Different possible causes of MTHF had been considered, documented or excluded. The role of alcohol was mainly as a "facilitator" and not definitely determinant as a single factor in causing the MTHF episode. The registry included patients with MTHF characterized by: decreased albumin levels; increased total bilirubin; altered hepatic functions enzymes; increased oxidative stress. Two management groups were created: a. standard management (SM) only; b: SM and Liverubin; 25 Liverubin patients and 23 SM subjects completed the registry. The average follow-up period was 32.2;1.3 days in the supplement group and 32.1;2 days in controls.**
Results. **The distribution of symptoms and ultrasound results were comparable. Most symptoms observed at inclusion were disappeared or attenuated at 4 weeks in both groups. At inclusion, the values in the two groups were comparable. The increase in albumin levels was significantly (P<0.05 at 4 weeks) faster and the final values were higher in the Liverubin group. Total bilirubin was reduced in the supplement group better than in controls (P<0.05). Direct bilirubin values improved more in the supplement group at 4 weeks (P<0.05). The decrease of ALT-SGPT and AST-ASAT was more evident in the supplement group (P<0.05). Improvement in controls was more limited. Alkaline phosphatase value was normalized at 4 weeks in Liverubin patients; values decreased less in controls (P<0.05). Gamma GT decreased and were normal at 4 weeks with Liverubin. ESR was decreased in both groups (significantly more in the Liverubin group: P<0.05). There was a less important decrease in controls without normalization at 4 weeks. The white cell count was also better at 4 weeks in the supplement group; P<0.05). Plasma free radicals were significantly elevated in both groups at inclusion. A more significant decrease in the supplement group was observed at 4 weeks. Persisting, elevated values were seen in controls (P<0.05 in comparison with normal range). Platelets values improved in the Liverubin group (P<0.05) better than in controls. All other blood tests values (including hematocrit, renal function tests) were within the normal range at inclusion and at 4 weeks in both groups. Hepatitis markers were negative at inclusion and at 4 weeks. Compliance. Ninety-six percent of the Liverubin capsules were correctly used. Safety and tolerability were optimal (no side effect was registered).**
Conclusion. **In conclusion, data from this pilot, registry study indicate a significant activ-**

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ity of Liverubin associated with a very good safety profile, in patients with temporary hepatic failure. The recovery of hepatic function is faster and more effective with Liverubin compared to the best "standard" management.

KEY WORDS: Liver failure - Hepatitis - Silymarin - Liverubin - Hepatic enzymes - Liver - Jaundice - Bilirubin.

Hepatic insufficiency may be caused by several infective or non-infective causes including dietary elements, some drugs, alcohol or a combination of these elements. Mild, temporary hepatic failure (MTHF) may be completely asymptomatic or cause minimal signs and symptoms.[1-7]

This common clinical problem is very diffuse and, in case of repeated episodes may cause a chronic impairment in liver functions.

Eventually, when sustained and severe, even permanent, organic lesions and functions impairment are observed in a significant number of patients. Alcohol abuse is possibly the most common identified cause of liver disease.[1, 2]

Alcohol - according to dosage - is directly toxic to liver cells and can cause alcoholic hepatitis (AH) leading to MTHF. Eventually, with chronic abuse, several functional alterations may develop leading to cell necrosis, fat cell accumulation and to more severe organic changes. With drug-induced liver disease hepatic cells become temporarily inflamed or permanently damaged as a consequence of acute or chronic exposure to some drugs.[1, 2]

Commonly used drugs may cause damage after an excessive dose leading to liver functions alterations and cellular injury.[1, 7] Other drugs or medications may cause damage even at appropriate dosages. The rate of hepatic complication following the use of medications is probably high but most episodes may be completely subclinical and undiagnosed.[1, 7] Methotrexate, used to treat autoimmune diseases and some tumors, may cause significant liver damage and even chronic alterations leading to cirrhosis. Disulfiram (Antabuse) used to treat alcoholic addiction may also be a cause of hepatic impairment and failure.[4-6] Liver failure can be also caused by several anti-inflammatory compounds.[4-7] The damage may be more evident when hepatic alterations - including alcohol damage are already present. Statin and niacin, used to control cholesterol, may cause liver damage even at appropriate dosages.[1, 2]

Liver function enzymes may be altered in a significant percent of patients. Several other treatments, drugs and medications may cause liver impairment (*i.e.*, some antibiotics: tetracyclines, nitrofurantoin, isoniazid, amoxicillin, clavulanic acid). Phytoproducts and excessive amounts of vitamins can cause hepatitis, liver failure and cirrhosis (vitamin A, kavakava, ma-huang, and comfrey). Several mushrooms are extremely, acutely poisonous even at low quantities for hepatic cells. The hepatic damage may kill in hours.

In a large number of patients the damaging agent or situation causing MTHF is not easily identified or may be hidden by the patients.

Usually, the management of MTHF [4-7] is based on avoiding the agents and situations leading to the functional damage. Infections, particularly hepatitis, should be always excluded in any patients with MTHF. At the moment, there is no defined, standard treatment as the damaging agent, if and when known, may have damaged the liver with a wide range of dosages and metabolic combinations. Avoiding any further possible damaging agent or situations and physical or metabolic stress is a key factor in management. Hydration, appropriate diet and observation (repeating blood tests, particularly the enzymes associated with MTHF) define the possible management for MTHF. Some pharmacological agents have been used with different degrees of success (including steroids) but at the moment there is no therapeutic standard for this cluster of conditions.

N-acetylcysteine has been found to be beneficial in cases of undefined acute liver failure.[2-8] A recent study [8] has indicated a protective effect of oak wood extracts on

liver injury. Liverubin and silymarin have also been used in several clinical studies.[10-15] Liverubin70/140 (Alchem, India) is a recent, standardized formulation based on the synthesis of natural elements obtained from Milk Thistle seeds traditionally used in the past for the management of liver damage. Sylimarin has been extensively studies in clinical contexts and regularly used in many parts of the world.[10-15]

The aim of this pilot registry was to evaluate the evolution of MTHF in subjects using Liverubin over a 4 week period.

Materials and methods

Inclusion criteria

Patients with MTHF were studied – after informed consent in a registry study. In all registered subjects viral hepatitis markers were negative at inclusion. Different possible causes of MTHF had been considered (Table I), documented or excluded. The role of alcohol intake in these subjects was mainly associated to other factors as a "facilitator" and not definitely determinant as a single factor in causing the MTHF episode. The registry included patients with MTHF characterized by:

— decreased albumin levels;
— increased total bilirubin;
— altered hepatic function enzymes;
— increased oxidative stress.

Management of acute mild-moderate liver failure

For these patients the best management option is to avoid any further negative effects caused by drugs or other agents. Subjects are generally asked to keep an adequate diet, rest and sleep, good hydration and basically to improve lifestyle (particularly avoiding alcoholic drinks and anything that may further endanger the liver, including smoking).[2]

This open-label registry included patients with a laboratory diagnosis of acute, moderate hepatic insufficiency based on enzyme and blood tests patterns and on an accurate evaluation of the liver with high-resolution ultrasound that excluded anatomic and organic lesions.

Exclusion criteria

The BMI (body-mass index) was on average 25.4;1 excluding obese subjects. Subjects with a BMI >26 were excluded. Patients were also excluded from the registry in case of unstable, severe or rapidly progressing disease, renal disease if they had history of hepatitis, were infected with HIV, if they had any metabolic or clinical disorder requiring treatment including anemia and diabetes. Congenital liver abnormalities, unstable mood and attention disorders, addictions, significant medical history requiring treatment, including the use of barbiturates or antiepileptic medications were also causes of exclusion.

Signs/symptoms

Minor, vague signs/symptoms were observed at inclusion and were considered for inclusion in this registry (at least 3 symptoms should have been present in the week before inclusion) only in associations with laboratory alterations. Signs and symptoms

TABLE I.—*Possible associated causes for moderate liver failure.*

	Number of subjects	
	25 supplement	23 controls
- Antibiotics	3	2
- Combination with unknown drugs	3	3
- Exclusively alcohol intake (alcoholic hepatitis)	8/25	7/23
- Aspirin use	4	3
- Anti-inflammatory drugs	7	8

Note: Viral hepatitis was excluded.

variations were considered a secondary target evaluation as not all patients clearly show signs and symptoms. Tiredness, fatigue, change in urine color, malaise, nausea, vomiting, upper right quadrant tenderness or pain were variably present in the registry subjects. Ultrasound-documented mild liver enlargement and mild splenomegaly were also present in all included subjects (Table I).

Liverubin [9-14] was used at the dosage of two tablets (each equivalent to 140 mg) daily.

Plasma free radicals

Oxidative stress in plasma can be measured in minutes with a drop of blood using a method described and validated in several studies.[16-18] This standardized method evaluates reactive oxygen species in peripheral blood. Measurements are expressed in Carr Units.[18]

All blood tests were evaluated at inclusion and repeated at 4 weeks.

Supplement studies

These registry studies [19] aim to define the field of activity of supplements and possible preventive, preferably non-clinical applications. The best fields of application for supplements are preclinical, borderline applications or the supplementary management of risk conditions. Supplements unless there are specific claims are not generally used for treatment of signs/symptoms or clinical conditions. The aim of supplement studies is to produce supplementary data to be compared to "background" historical data (*i.e.*, based on the best available management for comparable subjects) or to other management plans. In this study the supplement was used according to the following rules:

1. the use of the supplement was suggested to the evaluation subjects; the supplement use was not prescribed but suggested as an option, possibly capable of improving the management of the risk condition;

2. the supplement was only used on top of what was considered the "standard" or best-management/care' available, for that condition, according to international guidelines;

3. the use of the supplement should not have interfered with any other treatment, management or preventive measure;

4. time: the period of follow-up was considered variable, according to the needs and availability of the patients or registry subjects. The observation period is therefore variable, not prefixed. Ideally, the supplement administration should be used as long as needed to see results or changes;

5. the type of evaluation for these studies is always a registry.

The evaluation of the compliance concerning the use of the supplement is a significant value indicating how many subjects are actually willing to use the product. In these supplement studies there is no defined group allocation, no randomization organized by the investigators. Subjects decide on the basis of the initial briefing the management group they want to join including the control (non-supplement) group. No placebo is used.

Open label

Patients are always informed about the supplement or any treatment and management measure. A possible placebo effect is carefully explained and considered.

Data and results are analyzed only after the observation period, ideally, when sufficient evidence is collected or when fund limitations would eventually stop the collection of the observations. The time needed to detect differences among group is also considered an evaluation target. In this type of studies control groups, if present, are not necessarily parallel.

Characteristics of this study

This study was basically a small, independent, pilot, registry study; the evaluation product was not prescribed but recommended.

EXTERNAL STUDY REVIEWERS

All results and data were evaluated by an external reviewing panel, not in contact with the registry patients.

SPONSORS/CRO

Commercial sponsorship from the producers of the tested supplement was not available.

CONFLICTS OF INTEREST

There was no conflict of interest. All subjects were fully informed and provided informed consent.

Safety and tolerability

Safety and tolerability were assessed by clinical evaluation and laboratory measurements. Occurring adverse experiences were monitored throughout the registry. All clinical adverse experiences were evaluated in terms of intensity (mild, moderate, or severe), also considering duration, seriousness, outcome, and relationship to the study drug.

Statistical analysis

Study sample sizes were determined (at least 20 subjects in each group) from the estimates on the basis of a previous comparable study. Clinical and blood tests data were considered as non-parametric. An analysis of covariance (ANCOVA) model was used. The Hodges-Lehman estimate of the median difference (value for subjects with organ impairment – value for healthy subjects) was computed for supplemented subjects versus the control, standard management, population with the corresponding 90% confidence intervals (CI). These CI were based on the test statistic used for the Wilcoxon test.

Results

Table I indicates the suspected causes of hepatic injuries deducted from the medical history. In the final registry there were usable data concerning 25 Liverubin patients and 23 comparable controls completing the full follow-up, registry period (Table II). The average follow-up period was between 32.2;1.3 days in the supplement group and 32.1;2 days in controls.

Signs/symptoms

Table II shows the distribution of the most common symptoms and signs

TABLE II.—*Details of the two management registry groups (A). Drop-outs were due to missed controls.*

A. Number	Group	Management		
25	LIVERUBIN(LR)	(age range 45-60; all males)	BEST MANAGEMENT + LR	0
23	CONTROLS	(ege range 45-61; all males)	BEST MANAGEMENT ONLY	3

B. Signs/symptoms at inclusion	25 LR SUBJECTS		23 CONTROLS	
	INCLUSION	4 WEEKS	INCLUSION	4 WEEKS
Tiredness	22/25	6/25	20/23	13/23
Fatigue	21/25	5/25	21/23	12/23
Change in urine color	22/25	1/25	20/23	3/23
Malaise	24/25	5/25	22/23	7/23
Nausea	14/25	2/25	14/23	8/23
Vomiting	13/25	0/25	12/23	2/23
INR normal	25/25	25/25	23/23	23/23
Upper right quadrant tenderness/pain	14/25	2/25	15/23	5/23
Ultrasound-documented				
Mild liver enlargement	23/25	14/25	22/23	15/23
Mild spenomegaly	8/25	4/25	7/23	6/23

Note: number of patients with the symptom out of the total group.

and ultrasound results. The two groups (Liverubin and best management and the best management only) were comparable at inclusion. Due to the mainly subclinical, mild from a symptomatic point of view, type of liver dysfunction the actual beginning of the functional hepatic alterations is generally difficult to define and to associate to very specific events causing the alterations (*i.e.*, drug administration or a combination drug-alcohol). At the moment there is not a specific clinical system to evaluate the actual beginning of hepatic alterations (*i.e.*, before subjects had experienced signs/symptoms that may led them to consultations). Actually, symptoms in most of these patients may be completely unnoticed in most clinical settings unless specific tests or focused attention reveal the condition.

Most signs/symptoms observed at inclusion were completely disappeared or greatly attenuated at 4 weeks in both groups. This indicates that the evaluation of these patients should mainly rely on blood tests, as symptoms are very subjective. The presence of clinical problems may be transient in mild-moderate hepatic insufficiency, often subclinical and tends to disappear in 1-2 weeks, in some cases even faster.

Table III shows the results of the blood tests at inclusion and at 4 weeks in the two groups. At inclusion, the values in the two groups were comparable. The increase in albumin levels was significantly (P<0.05 at 4 weeks) faster and the final values were higher in the Liverubin group.

Total bilirubin was reduced in the supplement group better than in controls (P<0.05). Direct bilirubin values improved more in the supplement group at 4 (P<0.05). The decrease of ALT-SGPT and AST-ASAT was more evident in the supplement group (P<0.05). Improvement in controls was more limited. Alkaline phosphatase value was normalized at 4 weeks in Liverubin patients; the test values decreased less in controls at 4 weeks (P<0.05). Gamma GT values also significantly decreased and were normalized at 4 weeks in the supple-

TABLE III.—*Results at 4 weeks.*

TESTS	Normal Range	Units	Groups	Results	
				On inclusion	4 weeks
1. Albumin	3.5 - 5.3	g/dL	LR	3.22;1.2	4.3;1*#
			CONTROLS	3.2;1.2	3.8;1.2*
2. Total BIL	0.2 - 1.2	mg/dL	LR	2.51;0.7	1.23;0.8*#
			CONTROLS	2.52;0.4	1.7;0.5*
3. Direct BIL	0.1 - 0.4		LR	0.1;0.2	0.22;0.3*#
			CONTROLS	0.11;0.1	0.14;0.1*
4. ALT SGPT	7 - 56	IU/L	LR	122;18	43;11*#
			CONTROLS	131;15	68;22*
5. AST ASAT (serum glutamic oxaloacetic transaminase SGOT)	5 - 47	IU/L	LR	132;20	51;11**#
			CONTROLS	131;19	88;13*
6. Alk phosph alp	30 - 120	IU/L	LR	190;25	111;13*
			CONTROLS	186;21	129;20*#
7. Gamma GT	0 - 42	IU/L	LR	97.2;13	41;10*#
			CONTROLS	104;12	54;13*
7. ESR	0 - 23	mm/h	LR	41.2;11	22.3;5.5*#
			CONTROLS	40;8.3	31;10*
8. White cell count			LR	11 333	7 326*#
			CONTROLS	11 481	8 868*
9. Platelets			LR	149 113*	198 658*#
			CONTROLS	138 213	139 582*
10. Ox stress	<310	Carr units	LR	459;44	356;23*#
			CONTROLS	468;38	4216;22*

Other tests: nucleotidase, coagulation, glucose, lactate dehydrogenase and hematocrit were normal at inclusion and at 4 weeks.
* P<0.05 in comparison with previous value; #= P<0.05: difference between groups at 4 weeks.

ment group. The ESR was decreased in both groups (significantly more in the Liverubin group (P<0.05). There was a less important decrease in controls without normalization at 4 weeks, with persisting higher values. The white cell count was also better at 4 weeks (with a larger decrease in count in the supplement group; P<0.05).

Oxidative stress

Plasma free radicals were significantly elevated in both groups at inclusion. A more significant decrease in the supplement group was observed at 4 weeks. Persisting elevated values were seen in controls were observed (P<0.05 in comparison with normal range). Also platelets values improved in the Liverubin group (P<0.05) better than in controls.

All other blood tests values (including hematocrit, renal function tests) were within the normal range at inclusion and at 4 weeks in both groups.

Hepatitis markers (negative at inclusion) were negative when repeated at 4 weeks in all subjects.

Compliance: 96% of the Liverubin capsules were correctly used within the 4 weeks of the follow up. Safety was optimal and no side effects were registered. Tolerability was optimal and no patient had to stop treatment before the planned 4 weeks of follow-up.

Discussion

Hepatic function alterations may be due to several, possible hepatotoxic elements. They are very common and often subclinical. Repeated damage or added damage (including the increase in oxidative stress) [19] from more than a single hepatotoxic compound or damage in predisposed individuals may cause severe functional and organic hepatic damage that may become evident and severe and permanent in very advanced stages of hepatic failure.[20-25] Hepatotoxicity – even mild-moderate, transient (present for less than 3 months) – and hepatic failure are expressed by the presence of significantly altered enzyme values.[22-25] Gamma glutamyl-transpeptidase (GGT) may be elevated even in minor, subclinical levels of liver dysfunction. It can also be helpful in identifying the cause of an isolated elevation in ALP (GGT is raised in chronic alcohol toxicity).[27]

The improvement in liver enzymes values can be considered a significant indication of a reduction in functional and organic liver damage and can be effectively used to monitor MTHF due to drug or alcohol toxicity.[22] MTHF alcoholic liver disease (ALD) is associated with the overproduction of pro-inflammatory cytokines (such as interleukin-1β [IL-I β], IL-1, IL-8 and IL- 6) that have a significant pathological role in the development of ALD. Monocytes and Kupffer cells are activated with an increase in vascular permeability, cellular necrosis or apoptosis of hepatocytes as an expression of adhesion molecules on endothelial cells and the activation and chemo-attraction of neutrophils and mononuclear cells. Alcohol damage increases the levels of IL-1β, IL-1, IL-6 and IL-8 in animal models.[27] The variations of γ-GT levels are important indicators of hepatic damage, and are usually used as sensitive marker in the diagnosis of hepatic diseases.

In the present study, the serum levels γ-GT – clearly elevated at inclusion – were significantly reduced with Liverubin administration.

Silymarin extracts have already shown protective effect on liver injury in subjects with several types of hepatic failure.[10-15] Liverubin is a highly standardized extract and is made water-soluble by a specific patented process. Controlled laboratory tests have shown that the bioavailability of the product is 2-3 times higher than other silymarin preparations. The effects of Liverubin in MTHF are most probably the result of a significant, acute, anti-inflammatory action.[28]

Supplements, evaluated in an open registry including the best present management versus the best management plus the supplement, could be an important possible

solution to preliminarily evaluate possible management solutions to common clinical problems that do not have a defined therapeutic solution at the moment.

Conclusions

In conclusion, data from this pilot registry study indicate a significant potential activity of Liverubin associated with a very good safety profile, in patients with temporary hepatic failure. The recovery of hepatic function is faster and more effective with Liverubin compared to the best 'standard' management. The activity of Liverubin seems to be mediated - at least partially - by its strong antioxidant activity in subjects with increased oxidative stress.

Standard treatment is still lacking a defined management and treatments are generally undefined in mild hepatic failure.[29] In this clinical condition, an important new role can be assigned to the monitoring of white cells and platelet count (particularly in cases associated with alcoholic hepatitis and in jaundiced subjects).[30]

Larger and more prolonged registries and specific clinical trials may follow to better specify claims of this important supplementary treatment.

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