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Evaluation of efficacy and safety of Liv.52 DS tablets in acute viral hepatitis: A prospective, double-blind, randomized, placebo-controlled, phase III clinical trial

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ABSTRACT

Viral hepatitis (A, B, C, D, E and G) is a global public health problem, which is responsible for a major chunk of morbidity and mortality. Viral hepatitis occurs endemically and sporadically throughout the world, depending on the endemicity of infection. The primary goal in the management of acute viral hepatitis is early renormalization of hepatic functions with symptomatic and clinical recovery. This study was planned to evaluate the efficacy and safety of Liv.52 DS tablets in acute viral hepatitis.

This study was a prospective, double-blind, randomized, placebo-controlled, phase III clinical trial conducted as per the Declaration of Helsinki, with strict adherence with the GCP ethical guidelines and was approved by the institutional ethics committee. A total of 50 patients with diagnosis of symptomatic acute viral hepatitis, and who were willing to give informed consent were included in the study. Pregnant women, patients with chronic hepatitis, patients with malignant jaundice, patients with other causes for acute hepatitis, and those who were unwilling to give informed consent were excluded from the study.

At the initial visit, informed written consent was obtained from all the enrolled patients, and randomization and double blinding was done. A detailed medical history was

ABBREVIATIONS

ALP	: Alkaline phosphatase
ALT	: Alanine aminotransferase
AST	: Aspartate aminotransferase
CBC	: Complete blood count
CCl_4	: Carbon tetrachloride
DLC	: Differential leucocyte count
DNA	: Deoxyribonucleic acid
ESR	: Erythrocyte sedimentation rate
ET-NANBH	: Enterically transmitted non-A non-B hepatitis
GCP	: Good clinical practice
HA	: Hepatitis A
HAV	: Hepatitis A virus
Hb	: Hemoglobin
HBC	: Hepatitis B core (antigen)
HBeAg	: Hepatitis Be antigen
HBsAg	: Hepatitis B surface antigen
HBV	: Hepatitis B virus
HCV	: Hepatitis C virus
HDV	: Hepatitis D virus
HEV	: Hepatitis E virus
HGBV-C/ GB-C	: Hepatitis GB virus-C
HGV	: Hepatitis G virus
IgG	: Immunoglobulin G
IgM	: Immunoglobulin M
LFT	: Liver function test
PC	: Platelet count
RNA	: Ribonucleic acid
SA	: Serum albumin
SB	: Serum bilirubin
SG	: Serum glutathione
SGOT	: Serum glutamic oxaloacetic transaminase
SGPT	: Serum glutamic pyruvic transaminase
TB	: Total bilirubin
TLC	: Total leucocyte count
TP	: Total protein
WBC	: White blood cells

obtained from all the enrolled patients, which was followed by thorough clinical examination. All patients were subjected to hematological and biochemical investigations, which included CBC, LFTs, ESR and tests for viral markers (IgM antiHAV, IgM antiHBc, HBsAg, IgM antiHEV). The drug group received Liv.52 DS and the other group received placebo, in a dose of 2 tablets two times-a-day, orally, for 4 months. Patients were not allowed to take any other medication, which would have any significant effect on LFT. All patients were followed up every month for a period of 4 months. At each follow-up visit, the investigator recorded any information about adverse events, and a symptomatic evaluation was conducted, which was followed by thorough clinical examination. The subjective symptomatic improvement was assessed on a predefined 0 to 3 score scale.

At the end of 4 months, changes in the hematological and biochemical parameters from baseline values to the values at the end of the study, the duration of recovery, incidence of adverse events and patient compliance to the drug treatment were recorded. All adverse events either reported or observed by patients were recorded with information about severity, duration and action taken regarding the study drug. The predefined primary efficacy endpoints were rapid symptomatic improvement, renormalization of hematological and biochemical parameters, and total duration of clinical recovery. The predefined secondary safety endpoints were incidence of adverse events during the study period and overall compliance to the drug treatment. Statistical analysis was done according to intention-to-treat principles. The minimum level of significance was fixed at 99% confidence limit and a 2-sided p value of <0.001 was considered significant.

A total of 50 patients were enrolled in the trial. The demographic and clinical profile, hematological and biochemical profile were similar in Liv.52 DS and placebo groups and the patients were equally distributed in both the study arms. The mean age of the enrolled patients was 33.42 years. The common symptoms reported by patients were jaundice, anorexia, nausea, vomiting, fever and pruritus. The common clinical findings were icterus and hepatomegaly. Laboratory investigations confirmed that 74% patients were suffering from hepatitis E, 10% from hepatitis B, 8% from hepatitis A, and 8% from hepatitis non A-E. There was a highly significant ($p<0.0001$) and rapid symptomatic improvement in the mean scores for loss of appetite, weight loss, fatigue and jaundice in Liv.52 DS group, as compared to the placebo. There was a significant renormalization of the biochemical parameters of liver functions, after 2 months in Liv.52 DS group and after 3 months in the placebo group. There was a highly significant ($p<0.0001$) improvement in the blood proteins, and the levels of SA, SG and TP were renormalized. The increased level of ESR and WBC were significantly ($p<0.0001$) reduced in Liv.52 DS group, as compared to the placebo group, at the end of the study. There were no clinically significant ($p<0.0001$) changes in other biochemical parameters such as Hb levels and PC. There were no clinically significant adverse effects during the entire study period and the overall compliance to the drug treatment was excellent.

Therefore, it may be concluded that, Liv.52 DS tablets are clinically effective and safe in the management of acute viral hepatitis.

INTRODUCTION

Viral hepatitis (A, B, C, D, E and G) is a global public health problem, which is responsible for a major chunk of morbidity and mortality. Viral hepatitis occurs endemically and sporadically throughout the world, depending on the endemicity of infection. Based on the prevalence of infection, various regions of world have been classified into areas of high endemicity (8% and above - Africa, South East Asia, South America and Middle East), areas

of intermediate endemicity (2% to 8% - Australia, USA and Western Europe) and areas of low endemicity (below 2% - Northern Europe and Japan).

Hepatitis A is an acute, but benign form of viral hepatitis and is the least serious (self-limiting) disease. Acute hepatitis B can range from subclinical disease to fulminant hepatic failure and individuals with chronic hepatitis B are at increased risk for the development of hepatocellular carcinoma. Despite the discovery of hepatitis C, a permissive cell culture system for propagating HCV has yet to be established and a non-primate animal model does not exist. As a result, the production of specific drugs against HCV has been impeded although excellent diagnostic methods have been developed. The HDV infection occurs only in the presence of hepatitis B infection (co-infection or super-infection). Hepatitis E virus is ET-NANBH, which is clinically indistinguishable from hepatitis A disease and the disease is usually is mild expect in pregnant women, who appear to be exceptionally susceptible to severe disease and excessive mortality. The HGV/GBV-C is a recently characterized flavivirus, that may cause acute and chronic hepatitis and the precise role of HGV/GBV-C in human disease is currently under investigation.

The primary goals for managing acute viral hepatitis are to provide adequate nutrition (with restricted protein and fat intake), to prevent further damage to the liver, and to prevent transmission of infection to others. Therefore, early renormalizations of hepatic functions with symptomatic and clinical recovery are the primary objectives in the clinical management of acute hepatitis. There are no established drugs for hepatitis A, hepatitis E and hepatitis G. However, the available treatment options for hepatitis B, hepatitis C and hepatitis D have various limitations, such as dependable clinical efficacy, associated adverse effects and affordability issues.

Liv.52 DS tablet is a polyherbal formulation, which have been used extensively in the management of HA. Each Liv.52 DS tablet contains powders of *Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium, Tamarix gallica* and Mandura bhasma**.** This study was planned to evaluate the efficacy and safety of Liv.52 DS tablets in acute viral hepatitis.

Aim of study

The study was planned to evaluate the clinical efficacy, and short- and long-term safety of Liv.52 DS tablets in acute viral hepatitis.

Study design

This study was a prospective, double blind, randomized, placebo-controlled, phase III clinical trial conducted at Jagjivanram Western Railway Hospital, Mumbai, India, from February 2003 to February 2004, as per the Declaration of Helsinki, with strict adherence with the GCP ethical guidelines. The study protocol, case report forms, regulatory clearance documents, product related information and informed consent form (in Bengali and English) were submitted to the Institutional Ethics Committee, and were approved by the same.

MATERIALS AND METHODS

Inclusion criteria

A total of 50 patients with diagnosis of symptomatic acute viral hepatitis, and who were willing to give informed consent were included in the study.

Exclusion criteria
Pregnant women, patients with chronic hepatitis (more than 6 months), patients with malignant jaundice, patients with other causes for acute hepatitis (drug-induced), and those who were unwilling to give informed consent were excluded from the study.

Study procedure
At the initial visit, informed written consent was obtained from all the enrolled patients, after explaining to them the nature of the study; after which randomization and double blinding was done. A person unconnected with the study did randomization by using computer generated random number allocation and each arm of the study had 25 patients. Due to double blinding procedure, neither the patient nor the investigators were aware of the drug identity and therefore were unable to predict the treatment allocation. The drug identity codes were kept in sealed envelopes at a secure location to ensure the double blinding of treatment allocation.

A detailed medical history was obtained from all enrolled patients, which was followed by thorough clinical examination. All patients were subjected to hematological and biochemical investigations, which included CBC (Hb, TLC, DLC, PC), LFTs (SGOT, SGPT, SB, SA, SG, TP), ESR and tests for viral markers (IgM antiHAV, IgM antiHBc, HBsAg, IgM antiHEV).

Study drugs
The daily dose of 2 tablets of Liv.52 DS has been shown to be potentially effective and safe in the management of acute viral hepatitis and was considered adequate for this study. The drug group received Liv.52 DS and the other group received placebo. Both groups were advised to consume the drugs in doses of 2 tablets two times-a-day, orally, for 4 months. Patients were not allowed to take any other medication, which would have any significant effect on LFT.

Follow-up and monitoring
All patients were followed up every month for a period of 4 months. At each follow-up visit (1^{st}, 2^{nd}, 3^{rd} and 4^{th} month), the investigator recorded any information about adverse events (either reported or observed), and symptomatic evaluation ws conducted, which was followed by thorough clinical examination. The subjective symptomatic improvement (jaundice, anorexia, nausea, vomiting, fever and pruritus) was assessed on a predefined 0 to 3 score scale (0=poor, 1=average, 2=good, 3=excellent). At the end of 4 months, changes in the hematological and biochemical parameters from baseline values to the values at the end of the study, the duration of recovery (symptomatic, hematological, biochemical and clinical), incidence of adverse events (either reported or observed) and patient compliance to the drug treatment were recorded.

Adverse events
All adverse events either reported or observed by patients were recorded with information about severity, duration and action taken regarding the study drug. Relation of adverse events to the study medication was predefined as *"Unrelated"* (a reaction that 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).

For patients recorded as withdrawing from the study, efforts were made to ascertain the reason for dropout. Non-compliance (defined as failure to take less than 80% of the medication) was not regarded as treatment failure, and reasons for non-compliance were recorded.

Primary and secondary endpoints

The predefined primary efficacy endpoints were rapid symptomatic improvement, renormalization of hematological and biochemical parameters, and total duration of clinical recovery. The predefined secondary safety endpoints were incidence of adverse events (either reported or observed) during the study period and overall compliance to the drug treatment.

Statistical analysis

Statistical analysis was done according to intention-to-treat principles. *"Repeated Measures ANOVA Test"* and *"Bonferroni's Multiple Comparison Test"* analyzed the mean score for monthly symptomatic improvement. Changes in various parameters from baseline values and values at the end of the study were pooled and analyzed cumulatively by *"Paired 't' Test"*. The minimum level of significance was fixed at 99% confidence limit and a 2-sided *p* value of <0.001 was considered significant.

RESULTS

A total of 50 patients were enrolled in this trial and there was male preponderance in the study population (38 males and 12 females). The demographic profile, clinical profile, hematological and biochemical profile were similar in the Liv.52 DS and placebo groups and the patients were equally distributed in both the study arms.

The mean age of the enrolled patients was 33.42 years (minimum=12, maximum=63, SD=13.76, SEM=1.95, Lower 99% CI of M=28.19 and Upper 99% CI of M=38.65). The common symptoms reported by patients were jaundice (100%), anorexia (80%), nausea (74%), vomiting (40%), fever (28%) and pruritus (2%). The common clinical findings were icterus (100%) and hepatomegaly (36%). Laboratory investigations confirmed that 74% patients were suffering from hepatitis E, 10% from hepatitis B, 8% from hepatitis A, and 8% from hepatitis non A-E.

There was a highly significant ($p<0.0001$) and rapid symptomatic improvement in the mean scores for loss of appetite, weight loss, fatigue and jaundice in the Liv.52 DS group, as compared to the placebo. In the Liv.52 DS group, significant ($p<0.0001$) clinical improvement in loss of appetite was observed over a period of 1 month and total renormalization of appetite was evident by 2 months, while in the placebo group renormalization of appetite was seen only after 3 months (Table 1). Liv.52 DS controlled weight loss significantly ($p<0.0001$) in a month and weight gain was evident in 2 months, while in the placebo group weight gain was seen only after 3 months (Table 2). There was a highly significant ($p<0.0001$) improvement in fatigue in the Liv.52 DS group over a period of 1 month and complete improvement was observed by 3 months, while in the placebo group improvement was seen only after 3 months (Table 3). In the Liv.52 DS group, significant ($p<0.0001$) clinical improvement in jaundice was evident by 1 month and there was complete improvement in 2 months, while in the placebo group improvement was seen only after 3 months (Table 4 and Figure 1).

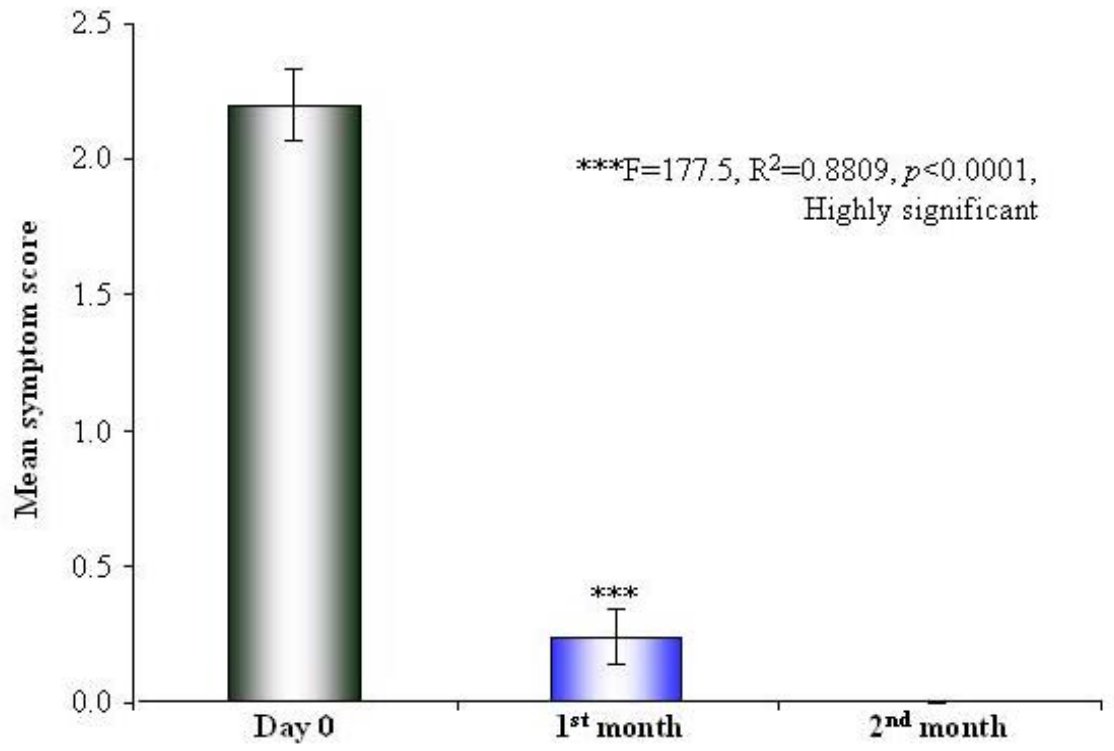
Table 1: Improvement in mean symptom score of loss of appetite				
Repeated Measures ANOVA Test				
Parameter	**Day 0**	**1st month**	**2nd month**	
Mean	2.32	0.68	0.00	
Std. Deviation	0.90	0.69	0.00	
Std. Error	0.18	0.14	0.00	
Lower 99% CI	1.82	0.29	0.00	
Upper 99% CI	2.82	1.07	0.00	
Significance	***F=109.9, R^2=0.8208, p<0.0001, Highly Significant			
Bonferroni's Multiple Comparison Test				
	Mean Diff.	**t**	***p* value**	**99% CI of Diff.**
Day 0 vs 1st month	1.64	12.09	p<0.001	1.219 to 2.06
1st month vs 2nd month	1.28	8.01	p<0.001	0.258 to 1.101

Table 2: Improvement in mean symptom score of weight loss				
Repeated Measures ANOVA Test				
Parameter	**Day 0**	**1st month**	**2nd month**	
Mean	0.56	0.04	0.00	
Std. Deviation	0.87	0.20	0.00	
Std. Error	0.17	0.04	0.00	
Lower 99% CI	0.07	-0.07	0.00	
Upper 99% CI	1.05	0.15	0.00	
Significance	***F=110.19, R^2=0.298, p<0.0001, Highly significant			
Bonferroni's Multiple Comparison Test				
	Mean Diff.	***t***	***p* value**	**99% CI of Diff.**
Day 0 vs 1st month	0.52	4.76	P<0.001	0.180 to 0.859
1st month vs 2nd month	0.04	0.36	p>0.05	-0.299 to 0.379
2nd month vs 3rd month	0	0	p>0.05	-0.339 to 0.339

Table 3: Improvement in mean symptom score of fatigue					
Repeated Measures ANOVA Test					
Parameter	**Day 0**	**1st month**	**2nd month**	**3rd month**	
Mean	2.52	1.52	1.00	0.00	
Std. Deviation	0.51	0.51	0.00	0.00	
Std. Error	0.10	0.10	0.00	0.00	
Lower 99% CI	2.24	1.24	1.00	0.00	
Upper 99% CI	2.81	1.81	1.00	0.00	
Significance	***F=367, R^2 =0.9386, p<0.0001, Highly significant				
Bonferroni's Multiple Comparison Test					
	Mean Diff.	***t***	***p* value**	**99% CI of Diff.**	
Day 0 vs 1st month	1	12.66	p<0.001	0.754 to 1.245	
1st month vs 2nd month	0.52	6.58	p<0.001	0.274 to 0.765	
2nd month vs 3rd month	0	0	p>0.05	-0.245 to 0.245	

Table 4: Clinical Improvement in mean symptom score of jaundice				
Repeated Measures ANOVA Test				
Parameter	**Day 0**	**1st month**	**2nd month**	
Mean	2.20	0.24	0.00	
Std. Deviation	0.65	0.52	0.00	
Std. Error	0.13	0.10	0.00	
Lower 99% CI	1.84	-0.05	0.00	
Upper 99% CI	2.56	0.53	0.00	
Significance	***F=177.5, R^2=0.8809, p<0.0001, Highly significant			
Bonferroni's Multiple Comparison Test				
	Mean Diff.	***t***	***p* value**	**99% CI of Diff.**
Day 0 vs 1st month	1.96	19.18	p<0.001	1.643 to 2.277
1st month vs 2nd month	1.67	12.38	p<0.001	0.077 to 0.553
2nd month vs 3rd month	0.00	0.00		

Figure 1: Clinical improvement in mean symptom score of jaundice at Day 0, 1st month and 2nd month with Liv.52 DS treatment



There was significant renormalization of the biochemical parameters of liver functions after 2 months in the Liv.52 DS group and after 3 months in the placebo group. There was a highly significant (p<0.0001) reduction in the levels of SGOT (p<0.0001) (Table 5 and Figure 2), SGPT (p<0.0001) (Table 6 and Figure 3) and SB (p=0.0008) (Table 7 and Figure 4) in the Liv.52 DS group. There was highly significant improvement in the blood proteins, and the levels of SA (p<0.0001) (Table 8), SG (p=0.0083) (Table 9) and TP (Table 10) were renormalized. The elevated levels of ESR (p=0.002) (Table 11 and Figure 5) and WBC ($p=0.0412$) (Table 12) were significantly reduced in the Liv.52 DS group, as compared to the

placebo group, at the end of the study. There were no clinically significant changes in other biochemical parameters as Hb levels (Table 13) and PC (Table 14).

Table 5: Improvement in SGOT (IU/L) levels at baseline and after 4th month with Liv.52 DS treatment

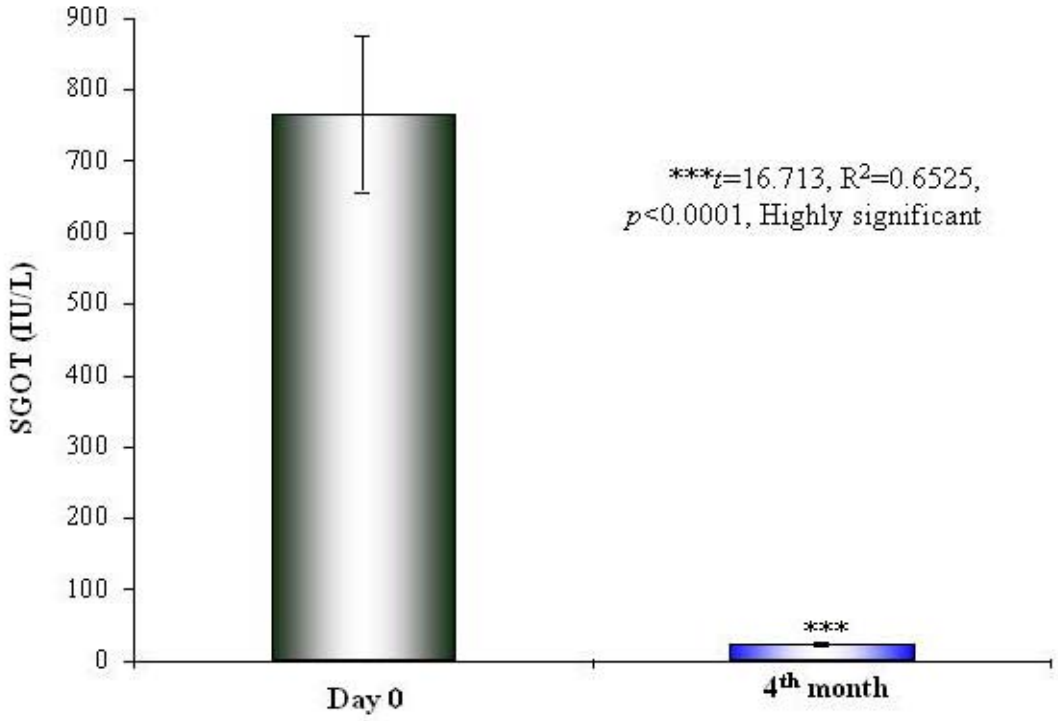
Parameter	Day 0	4th month
Minimum	139.00	10.00
Maximum	2000.00	42.00
Mean	767.40	23.56
Std. Deviation	550.60	10.68
Std. Error	110.10	2.14
Lower 99% CI	459.40	17.59
Upper 99% CI	1075.00	29.53
Mean of differences	743.9	
99% CI	433.9 to 1054	
R^2	0.6525	
t value	16.713	
p value	$p<0.0001$	
p value summary	***Highly Significant	

There were no clinically significant adverse effects, either observed or reported; during the entire study period, and the overall compliance to the drug treatment was found to be excellent.

DISCUSSION

Hepatitis A is an acute, but benign form of viral hepatitis caused by an RNA virus and the virus does not persist in the blood serum. Hepatitis A virus (HAV) is an enterovirus group of the picornaviridae family and HAV has a single molecule of RNA surrounded by a small (27 nm diameter) protein capsid. Hepatitis A is a food/waterborne disease, with feco-oral transmission. The incubation period is variable (from 15 to 25 days) and is

Figure 2: Improvement in SGOT levels at Day 0 and 4th month with Liv.52 DS treatment



inversely proportional to infective dose (presumably 10-100 virus particles). Hepatitis A is usually a mild illness characterized by sudden onset of fever, malaise, nausea, anorexia and abdominal discomfort, followed in several days by jaundice. The dark urine (which precedes jaundice by $^2/_3$ days) indicates the onset of the disease. The risk of transmission is generally low amongst household contacts, but outbreaks occur in nurseries and institutions with attack rates around 10%-15%. The duration of viral shedding is upto 14 days; with a sharp fall off after 5 days, but the period of infectiousness is about 8 to 17 days (the stool remains infectious upto 15 days). Asymptomatic infections are frequent. The serial interval between

index and secondary cases is either shorter or equal to incubation period, indicating that transmission usually occurs before or around onset of jaundice.[1]

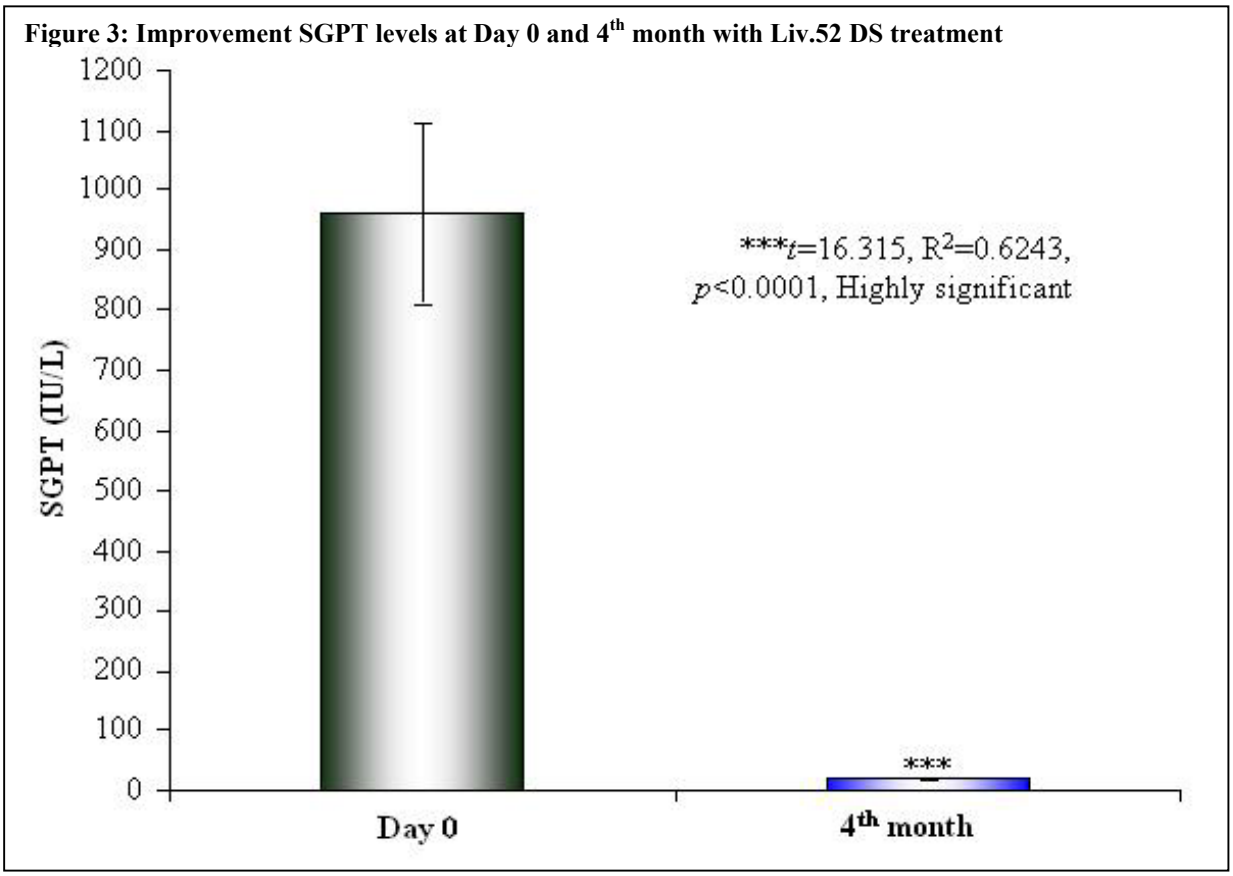
Table 6: Improvement in SGPT (IU/L) levels at baseline and after 4th month with Liv.52 DS treatment

Parameter	Day 0	4th month
Minimum	192.00	12.00
Maximum	2430.00	38.00
Mean	960.90	21.52
Std. Deviation	742.10	8.07
Std. Error	148.40	1.61
Lower 99% CI	545.80	17.01
Upper 99% CI	1376.00	26.03
Mean of differences	939.40	
99% CI	523.3 to 1355	
R^2	0.6243	
t value	16.315	
p value	$p<0.0001$	
p value summary	***Highly Significant	

Hepatitis A virus is diagnosed by finding IgM anti-HAV in serum during the acute or early convalescent phase of disease. Moderate increase in conjugated bilirubin, with marked elevation of SGOT, SGPT and lactate dehydrogenase isoenzymes are noted in HA (low Na^+ and Cl^- levels may be seen due to severe vomiting). Marginal elevation of ALP is seen due to compression of intrahepatic biliary canaliculi by swollen parenchymatous cells. Triglycerides and cholesterol may be temporarily and marginally lowered. Hepatitis A virus is the least serious of the hepatitis viruses, as it does not kill liver cells and also, there is no risk of a chronic form. Hepatitis is a self-limiting disease and no specific treatment is available.[1]

The HBV is a double-stranded DNA virus in the hepadnaviridae family. Hepatitis B virus is transmitted horizontally (by blood, blood products and sexual transmission) and vertically (from mother to infant in the perinatal period, which is a major mode of transmission in endemic regions). Hepatitis B virus causes acute and chronic hepatitis and the chances of becoming chronically infected depend upon age (about 90% of infected neonates and 50% of

Figure 3: Improvement SGPT levels at Day 0 and 4th month with Liv.52 DS treatment



infected young children will become chronically infected and in contrast, only about 5% to 10% of immunocompetent adults infected with HBV develop chronic hepatitis B). Acute hepatitis B can range from subclinical disease to fulminant hepatic failure and about 90% of acutely infected adults recover without sequelae, while about 5% of acutely infected adults become chronically infected.

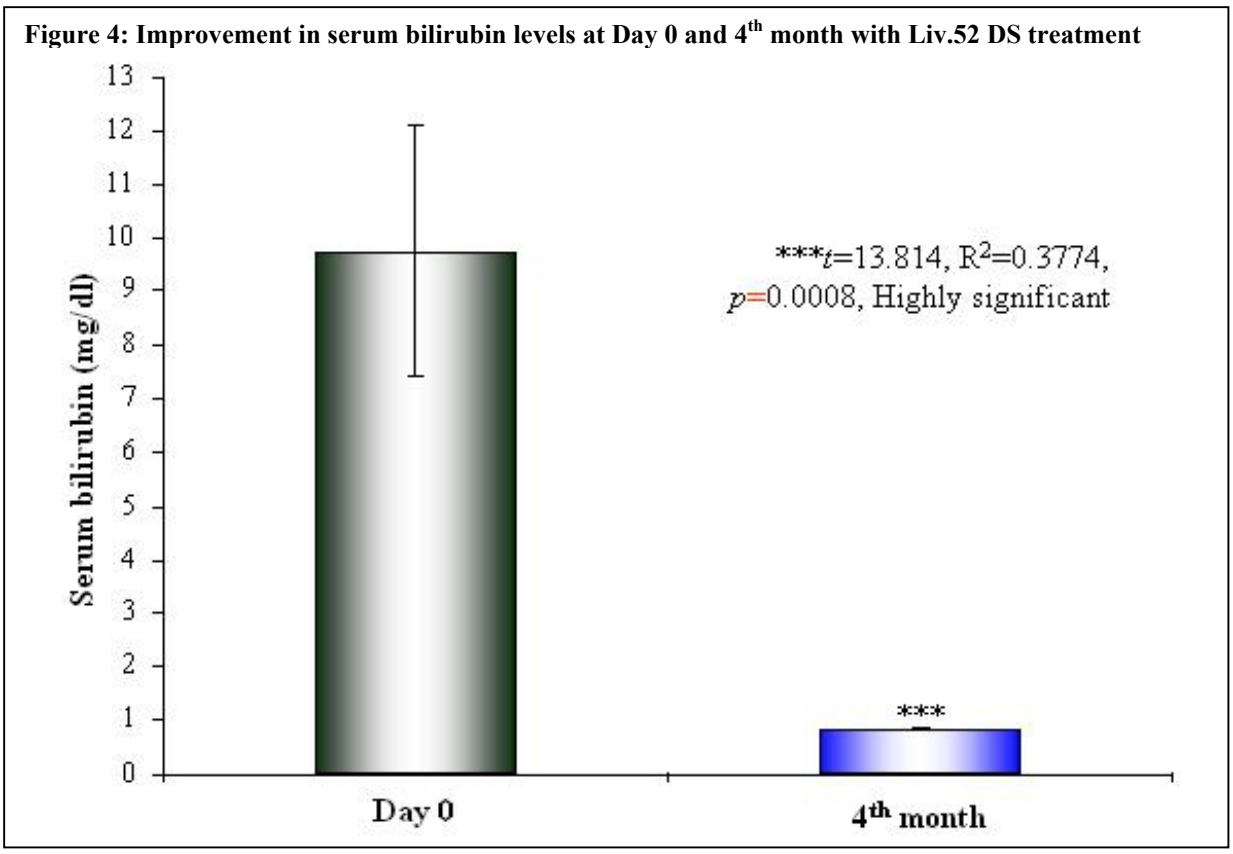
Table 7: Improvement in serum bilirubin (mg/dl) levels at baseline and after 4th month with Liv.52 DS treatment

Parameter	Day 0	4th month
Minimum	1.00	0.50
Maximum	61.17	1.10
Mean	9.76	0.83
Std. Deviation	11.72	0.16
Std. Error	2.34	0.03
Lower 99% CI	3.20	0.74
Upper 99% CI	16.31	0.92
Mean of differences	8.93	
99% CI	2.380 to 15.47	
R^2	0.3774	
t value	13.814	
p value	*p*=0.0008	
p value summary	***Highly significant	

Chronic infection with HBV can be either "replicative" or "non-replicative." In non-replicative infection, the rate of viral replication in the liver is low and serum HBV DNA concentration is low and HBeAg is not detected. In "replicative" infection, the patient usually has a relatively high serum concentration of viral DNA and detectable HBeAg. Patients with chronic hepatitis B and "replicative" infection have a worse prognosis and a greater chance of developing cirrhosis and/or hepatocellular carcinoma.

Virtually all individuals infected with HBV will have detectable serum HBsAg. In acute infection, HBsAg is detectable several weeks after infection and its appearance coincides with the onset of clinical symptoms. At around the same time, IgM antibodies against core antigen are detectable in serum and subsequently, IgG antibodies against core are produced. As acute infection resolves, IgG antibodies against core antigen persist and IgM antibodies and HBsAg become undetectable. Most people who have had acute infection that resolves continue to have IgG antibodies against core antigen for life. Acutely infected individuals

Figure 4: Improvement in serum bilirubin levels at Day 0 and 4th month with Liv.52 DS treatment



who do not clear HBV continue to have serum HBsAg. In most cases, the chronic infection becomes "non-replicative" and the subjects lose serum HBeAg and develop antibodies against HBeAg. In some cases, "replicative" infection persists along with detectable serum HBeAg. In chronically infected individuals, infection can switch from "non-replicative" to "replicative" and vice-versa. One goal of treatment is to convert patients with chronic hepatitis B from a "replicative" (HBeAg positive) to "non-replicative" (HBeAg negative) state. Individuals with chronic hepatitis B are at increased risk for the development of hepatocellular carcinoma, and should be screened by serum alpha-fetoprotein and ultrasonography examination.

Table 8: Improvement in serum albumin (gm/dl) levels at baseline and after 4th month

Parameter	Liv.52 DS		Placebo	
	Day 0	4th month	Day 0	4th month
Minimum	3.60	3.50	3.50	3.80
Maximum	4.90	4.75	4.79	4.70
Mean	4.38	4.28	4.32	4.32
Std. Deviation	0.28	0.28	0.29	0.25
Std. Error	0.06	0.06	0.06	0.05
Lower 99% CI	4.22	4.13	4.15	4.17
Upper 99% CI	4.54	4.44	4.49	4.47
Mean of differences	0.0968		*-0.0056	
99% CI	0.0313 to 0.1623		*-0.2018 to 0.1905	
R^2	0.4162		0.0002	
t value	4.137		0.08123	
p value	*p*<0.0001		*p*=0.936	
p value summary	*** Highly Significant		Non-significant	

Table 9: Improvement in Serum globulin (gm/dl) levels at baseline and after 4th month

Parameter	Liv.52 DS		Placebo	
	Day 0	4th month	Day 0	4th month
Minimum	2.00	2.20	2.50	2.50
Maximum	4.50	4.80	4.30	4.10
Mean	3.48	3.75	3.48	3.54
Std. Deviation	0.60	0.52	0.42	0.56
Std. Error	0.12	0.10	0.08	0.11
Lower 99% CI	3.15	3.46	3.25	3.22
Upper 99% CI	3.82	4.04	3.72	3.85
Mean of differences	0.2656		-0.052	
99% CI	0.0072 to 0.5239		-0.4406 to 0.3366	
R^2	0.2563		0.0058	
t value	2.876		0.3743	
p value	*p*=0.0083		*p*=0.7115	
p value summary	*** Highly significant		Non-significant	

Prior to the discovery of HCV, hepatitis following blood transfusion that was not caused by hepatitis A or hepatitis B was referred to as non-A, non-B hepatitis. Hepatitis C virus is a single-stranded RNA virus in the flaviviridae family. Primarily blood, blood products and sexual transmission transmit the virus (perinatal transmission is relatively low). About 85% of individuals acutely infected with HCV become chronically infected and most instances of acute infection are clinically undetectable. The natural history of chronic HCV infection can vary dramatically between individuals. About 20% of individuals with hepatitis C develop cirrhosis, which leads to end-stage liver disease; and there is an increased risk of developing hepatocellular carcinoma. History, serological testing and liver biopsy make the diagnosis of chronic hepatitis C. The presence of anti-HCV antibodies in a person with a risk factor or evidence of liver disease suggests the diagnosis of chronic hepatitis C. Tests for HCV RNA in blood in those individuals with anti-HCV antibodies confirms the diagnosis.

The HDV (also called delta virus) is a small circular RNA virus and in humans, HDV infection only occurs in the presence of hepatitis B infection, as the HDV is replication defective and therefore cannot propagate in the absence of another virus. Hepatitis D virus infection is transmitted by blood and blood products; and the risk factors for infection

are similar to those for HDV infection. A patient can acquire co-infection with HDV and HBV; and a patient with hepatitis B can be infected with HDV at any time after acute hepatitis B virus infection (super-infection). Hepatitis D virus super-infection should be suspected in a patient with chronic hepatitis B whose condition suddenly worsens and a particularly aggressive acute hepatitis B infection could suggest hepatitis D co-infection. Co-infection or super-infection with HDV in a patient with hepatitis B is diagnosed by the presence of antibodies against the HDV and IgM antibodies indicate acute infection

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Table 10: Improvement in serum total proteins (gm/dl) levels at baseline and after 4th month

Parameter	Liv.52 DS		Placebo	
	Day 0	4th month	Day 0	4th month
Minimum	6.60	6.80	6.70	7.00
Maximum	9.00	9.00	8.60	8.30
Mean	7.88	8.11	7.87	7.71
Std. Deviation	0.60	0.61	0.47	0.38
Std. Error	0.12	0.12	0.09	0.08
Lower 99% CI	7.55	7.77	7.61	7.50
Upper 99% CI	8.22	8.45	8.13	7.92
Mean of differences	0.2288		0.1624	
99% CI	-0.0097 to 0.4674		-0.1733 to 0.4981	
R^2	0.2307		0.0708	
t value	2.683		1.353	
p value	*p*=0.013		*p*=0.1886	
p value summary	*** Highly significant		Non-significant	

Hepatitis E Virus is very labile RNA virus with a particle diameter of 32-34 nm and it causes ET-NANBH, which is clinically indistinguishable from hepatitis A disease. Diagnosis of HEV is based on the epidemiological characteristics of the outbreak and by exclusion of hepatitis A and B viruses by serological tests. Hepatitis E virus is transmitted by the fecal-oral and person-to-person spread. Hepatitis E occurs in both epidemic and sporadic-endemic forms and the incubation period for hepatitis E varies from 2 to 9 weeks. The disease usually is mild and resolves in 2 weeks, leaving no sequelae. Pregnant women appear to be exceptionally susceptible to severe disease, and excessive mortality has been reported in this group.

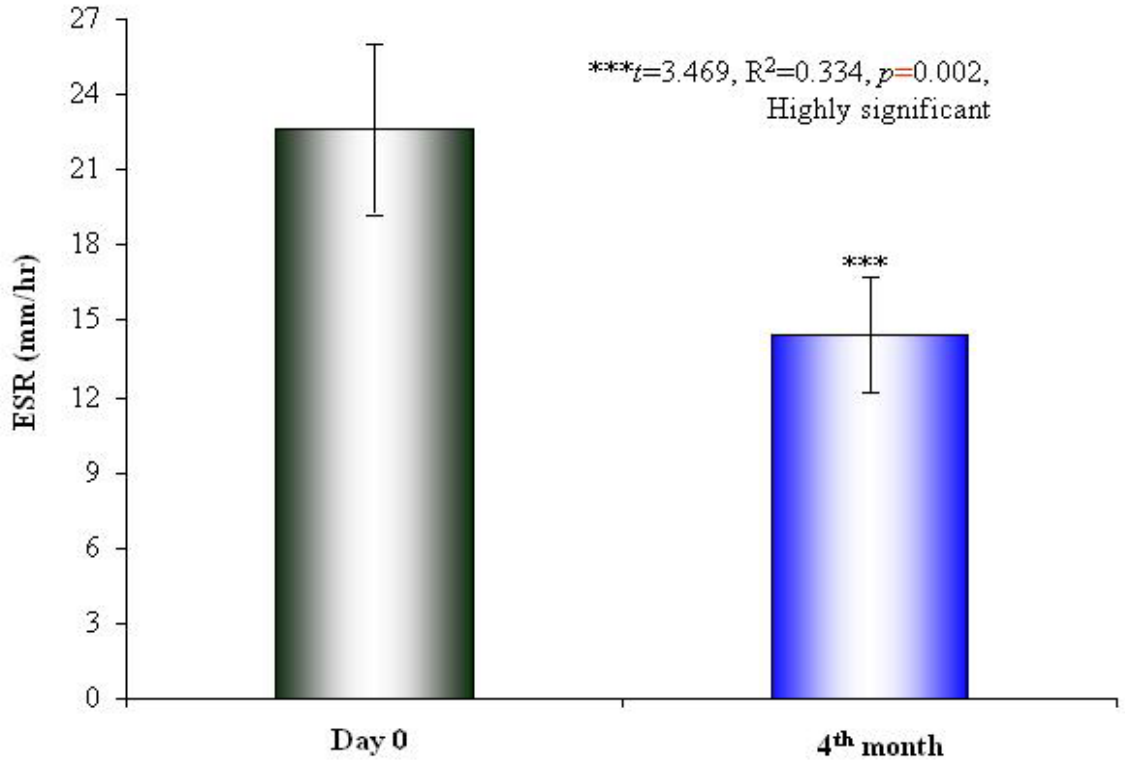
Table 11: Improvement in ESR (mm/hr) levels at baseline and after 4th month

Parameter	Liv.52 DS		Placebo	
	Day 0	4th month	Day 0	4th month
Minimum	5.00	5.00	2.00	2.00
Maximum	75.00	62.00	80.00	42.00
Mean	22.64	14.44	25.04	12.04
Std. Deviation	16.83	11.28	19.19	7.66
Std. Error	3.37	2.26	4.00	1.60
Lower 99% CI	13.22	8.13	13.76	7.54
Upper 99% CI	32.06	20.75	36.32	16.55
Mean of differences	8.2		13	
99% CI	1.589 to 14.81		114 to 23.89	
R^2	0.334		0.34	
t value	3.469		3.366	
p value	*p*=0.002		*p*=0.0028	
p value summary	*** Highly significant		Non-significant	

The HGV/HGBV-C is a recently characterized flavivirus (RNA virus) that may cause acute and chronic hepatitis. These new viruses are related to, but distinct from the flavivirus hepatitis C. Three viruses have been termed GB-A, GB-B and GB-C and based on genomic sequence comparisons; HGV is probably the same as GB-C. The precise role of HGV/GB-C in human disease is currently under investigation; however, most experts now feel that this virus is not responsible for clinically significant cases acute or chronic hepatitis.[2-6]

The enrolled patients in this study were suffering from different types of hepatitis (hepatitis A, hepatitis B, hepatitis E and hepatitis non A-E). While evaluating the clinical efficacy of Liv.52 DS in acute hepatitis, the primary end points were selected by their

Figure 5: Improvement in ESR levels at Day 0 and 4th month with Liv.52 DS treatment



specific significance in natural history of hepatitis. Most adult acquired liver diseases cause impairment in bilirubin secretion from liver, which cause elevation of SB in the blood and in acute liver disease, the SB is usually elevated relative to the severity of the acute process. Blood levels of SGOT (also referred as AST) and SGPT (also referred as ALT is elevated in all types of hepatitis (viral, alcoholic, drug-induced, etc.). Albumin and globulin are the major proteins synthesized in the liver, which circulate in the bloodstream, and low SA, SG, TP concentrations indicate poor liver function.

Table 12: Improvement in WBC (cells/cu.mm.) levels at baseline and after 4th month

Parameter	Liv.52 DS		Placebo	
	Day 0	4th month	Day 0	4th month
Minimum	4800	4000	4000	4000
Maximum	11900	10000	10000	10000
Mean	8057	7332	7378	7117
Std. Deviation	1673	1494	1632	1636
Std. Error	334.5	298.8	340.2	341.1
Lower 99% CI	7121	6496	6419	6156
Upper 99% CI	8992	8168	8337	8079
Mean of differences	724.8		260.9	
99% CI	-214.9 to 1665		-143.5 to 665.2	
R^2	0.1624		0.1307	
t value	2.157		1.819	
p value	*p*=0.0412		*p*=0.0826	
p value summary	*Significant		Non-significant	

This study observed a highly significant and rapid symptomatic improvement in the mean scores for loss of appetite, weight loss, fatigue and jaundice in Liv.52 DS group, as compared to the placebo. There was a significant and rapid renormalization in the biochemical parameters of liver functions (SGOT, SGPT, SB), and the levels of SA, SG, and TP were renormalized. The increased levels of ESR and WBC were significantly reduced in Liv.52 DS group, as compared to the placebo group, at the end of the study. There were no clinically significant changes in other biochemical parameters such as Hb levels and PC, which indicate excellent short- and long-term safety profile of Liv.52 DS**.** There were no clinically significant adverse effects, either

observed or reported; during the entire study period, and the overall compliance to the drug treatment was found to be excellent. These beneficial clinical efficacies of Liv.52 DS in acute viral hepatitis might be due to the synergistic action of its ingredients, which had been well documented in various experimental and clinical studies by various researchers.

Table 13: Improvement in serum hemoglobin (gm/dl) levels at baseline and after 4th month

Parameter	Liv.52 DS		Placebo	
	Day 0	4th month	Day 0	4th month
Minimum	8.6	10	9	10
Maximum	16.9	15.1	18.2	17.2
Mean	12.96	13.15	13.37	13.08
Std. Deviation	1.978	1.364	2.424	1.847
Std. Error	0.3957	0.2728	0.5054	0.3851
Lower 99% CI	11.85	12.39	11.95	11.99
Upper 99% CI	14.07	13.91	14.8	14.16
Mean of differences	0.192		0.295	
99% CI	-0.8016 to 0.4176		-0.2955 to 0.8868	
R^2	0.03133		0.08287	
t value	0.881		1.41	
p value	*p*=0.3871		*p*=0.1725	
p value summary	Non-significant		Non-significant	

Khanfar et al. isolated and identified the active ingredients of *Capparis spinosa* as beta-sitosterylglucoside-6'-octadecanoate and 3-methyl-2-butenyl-beta-glucoside.[7] p-Methoxy benzoic acid isolated from *Capparis spinosa* was found to possess potent hepatoprotective activity against CCl_4, paracetamol (*in vivo*) and thioacetamide, galactosamine (*in vitro*) induced hepatotoxicity.[8] Al-Said et al. demonstrated the strong anti-inflammatory activity of *Capparis spinosa*, which was comparable to oxyphenbutazone.[9-10] Bonina et al. documented a significant antioxidant activity of *Capparis spinosa* and also identified flavonols (kaempferol and quercetin derivatives) and hydroxycinnamic acids (caffeic acid, ferulic acid, p-cumaric acid, and cinnamic acid) as major antioxidants from *Capparis spinosa*.[11] In another study, Germano et al. observed the antioxidant activity of *Capparis spinosa* using tests such as lipid peroxidation, bleaching of free radicals and autoxidation of iron ions.[12] Mahasneh et al. observed potent antimicrobial and antifungal activity of *Capparis* spinosa.[13,14]

He et al. isolated 2,3,4,9-tetrahydro-1H-pyrido- (3,4-b) indole-3-carboxylic acid, azelaic acid and daucosterol as the major constituents of *Cichorium intybus*.[15] and Du et al. identified the other chemical constituents as alpha-amyrin, taraxerone, baurenyl acetate and beta-sitosterol.[16] Aktay et al. and Zafar et al. observed the hepatoprotective effect (confirmed by histopathological examination) of *Cichorium intybus* against CCl_4-induced hepatotoxicity and reported significant prevention of the elevation of malondialdehyde formation (plasma and hepatic) and enzyme levels (AST and ALT).[17,18] Ahmed et al. screened *Cichorium intybus* for antihepatotoxic activity and measured the degree of protection using biochemical parameters (AST, ALT, ALP and TP). Potent antihepatotoxic activity (comparable to the silymarin) was observed with almost complete normalization of the tissues (as neither fatty accumulation nor necrosis was observed on histopathological study).[19] Kim et al. studied the effects of *Cichorium intybus* on the immunotoxicity of ethanol and reported significant increase in the number of circulating leukocytes, the weights of concerned organs (liver, spleen and thymus), number of splenic plaque forming cells, hemagglutination titers and the secondary IgG antibody response. There were also significant increases in delayed-type hypersensitivity reaction, phagocytic activity, natural killer cell activity, cell proliferation and interferon gamma secretion.[20] Sultana et al. reported that the presence of *Cichorium intybus* in the

reaction mixture (containing calf thymus DNA and free radical generating system) protects DNA against oxidative damage to its deoxyribose sugar moiety.

Table 14: Total platelet count (cells) at baseline and after 4th month

Parameter	Liv.52 DS		Placebo	
	Day 0	4th month	Day 0	4th month
Minimum	150000	150000	140000	140000
Maximum	360000	360000	320000	320000
Mean	229200	243600	236400	240000
Std. Deviation	41120	34750	37740	36380
Std. Error	8224	6949	8046	7757
Lower 99% CI	206200	224200	213600	218000
Upper 99% CI	252200	263000	259100	262000
Mean of differences	-14400		-3636	
99% CI	-28390 to -407.5		-16340 to 9072	
R^2	0.2566		0.0303	
t value	2.878		0.8101	
p value	*p*=0.0083		*p*=0.427	
p value summary	Non-significant		Non-significant	

All these studies suggest that the observed hepatoprotective effect of *Cichorium intybus* might be due to its ability to suppress the oxidative degradation of DNA in the tissue debris.[21] El et al. and Papetti et al. documented the antioxidative activity (radical scavenging effects, inhibition of hydrogen peroxide, and iron chelation) of *Cichorium* intybus.[22,23] Gurbuz et al. observed significant cytoprotection against ethanol-induced damage and these results were further confirmed by using histopathological techniques.[24] Amirghofran et al. reported the capacity of *Cichorium intybus* to enhance the proliferation of lymphocytes after stimulation with the allogenic cells.[25] Kim et al. investigated the effect of *Cichorium intybus* on mast cell-mediated immediate type allergic reactions and observed inhibition of systemic anaphylactic reaction, reduction in the plasma histamine level.[26] Ikeda et al. identified saponins (nigrumnins I and II) as the active ingredients of *Solanum* nigrum.[27] *Solanum nigrum* was investigated for its hepatoprotective activity against CCl_4-induced hepatic damage and Raju et al. observed remarkable hepatoprotective activity confirmed by evaluated biochemical parameters (AST, ALT, ALP and TB).[28] Sultana et al. demonstrated that *Solanum nigrum* protect DNA against the oxidative damage and the results suggest that the observed hepatoprotective effect of *Solanum nigrum* might be due to the ability to suppress the oxidative degradation of DNA in the tissue debris.[29] Moundipa et al. studied the effects of *Solanum nigrum* on hepatotoxicity and reported increased activity of aminopyrine N-demethylase, uridine diphosphate glucuronyltransferase and glutathione S-transferase, without any alteration in levels of ALP, ALT and gamma-glutamyltransferase levels in the serum.[29] Son et al. reported *Solanum nigrum* as a potent scavenger of hydroxyl radicals and diphenylpicrylhydrazyl radicals.[30] Prashanth Kumar et al. tested *in vitro Solanum nigrum* for its cytoprotection (against gentamicin-induced toxicity) and observed significant inhibition of cytotoxicity, alongwith hydroxyl radical scavenging potential, which might be the mechanism of cytoprotection.[31] Similarly, Akhtar et al. observed gastric mucosal cytoprotection offered by *Solanum nigrum* against aspirin-induced gastric ulcers.[32] Qureshi et al. reported the antifungal activity of *Solanum* nigrum.[33]

Upadhyay et al. identified arjunetoside, oleanolic and arjunic acids as active ingredients from *Terminalia* arjuna.[34] Munasinghe et al. reported the potent antioxidant activity of *Terminalia arjuna*, which might be due to its effects on lipid peroxidation.[35] Ali et al. demonstrated that arjunaphthanoloside from *Terminalia arjuna* inhibits nitric oxide production[36] and terminoside A isolated from *Terminalia arjuna* decreases inducible nitric oxide synthase levels in lipopolysaccharide-stimulated peritoneal macrophages.[37] Cheng et al. observed potent antiviral activity by virtue of inhibition of viral attachment and penetration by

Terminalia arjuna.[38] Perumal Samy et al. demonstrated the potent antibacterial activity of *Terminalia* arjuna.[39]

Jafri et al. reported significant hepatoprotective effects of *Cassia occidentalis* in chemically induced liver damage.[40] Bin-Hafeez et al. showed that Cassia *occidentalis* modulates hepatic enzymes and provides hepatoprotection against induced immunosuppression.[41] Samy et al. reported antimicrobial properties of *Cassia occidentalis* comparable with standard reference antibiotics.[42] Perez et al. reported strong antibacterial activity of *Cassia occidentalis* against *Salmonella typhi*.[43] Tona et al. reported the inhibitory effect of *Cassia occidentalis* on *Plasmodium falciparum* growth.[44] Caceres et al. and Graham et al. observed the antifungal activity of *Cassia occidentalis*.[45,46]

Harnyk et al. documented the clinically beneficial effects of *Achillea millefolium* in the treatment of chronic hepatitis.[47] Krivenko et al. reported similar clinical improvements in chronic hepatocholecystitis and angiocholitis with *Achillea millefolium*.[48] Lin et al. observed anti-hepatoma activity of *Achillea millefolium*.[49] Candan et al. and Bezic et al. reported the antioxidant and antimicrobial activities of *Achillea millefolium*.[50,51]

Devarshi et al. studied Mandura bhasma for the hepatoprotective property in hepatitis induced by CCl_4 and observed prevention of CCl_4 mediated changes in the enzyme activities, which suggest the hepatoprotective role of Mandura bhasma.[52]

Therefore, as discussed above, these synergistic actions (hepatoprotective, antimicrobial, antioxidant and anti-inflammatory) exhibited by the ingredients of Liv.52 DS might explain the beneficial mechanism of action of Liv.52 DS in acute viral hepatitis.

CONCLUSION

Viral hepatitis (A, B, C, D, E and G) is a global public health problem, which is responsible for a major chunk of morbidity and mortality. Viral hepatitis occurs endemically and sporadically throughout the world, depending on the endemicity of infection. The primary goal in management of acute viral hepatitis is the early renormalizations of hepatic functions with symptomatic and clinical recovery. There are no established drugs for hepatitis A, hepatitis E and hepatitis G; while the available treatment options for hepatitis B, hepatitis C and hepatitis D have various limitations such as dependable clinical efficacy, associated adverse effects and affordability issues. This study was planned to evaluate the efficacy and safety of Liv.52 DS tablets in acute viral hepatitis.

The enrolled patients in this study were suffering from different types of hepatitis (hepatitis A, hepatitis B, hepatitis E and hepatitis non A-E). This study observed a highly significant and rapid symptomatic improvement in the mean scores for loss of appetite, weight loss, fatigue and jaundice in Liv.52 DS group. There was a significant and rapid renormalization in the biochemical parameters of liver functions and the levels of SA, SG, and TP were renormalized. The increased level of ESR and WBC were significantly reduced in Liv.52 DS group. There were no clinically significant changes in the other biochemical parameters such as Hb levels and PC, which indicates excellent short- and long-term safety profile of Liv.52 DS. There were no clinically significant adverse effects, either observed or reported during the entire study period, and the overall compliance to the drug treatment was found to be excellent. Therefore, it may be concluded that, Liv.52 DS tablets are clinically effective and safe in the management of acute viral hepatitis.

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