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Evaluation of safety and efficacy of a Polyherbal formulation Liv.52 DS in the Management of Non-Alcoholic Steatohepatitis (NASH): An open clinical study

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KEYWORDS

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Clinical study

A B S T R A C T

Non-alcoholic steatohepatitis (NASH) is a clinicopathological entity characterized by histological features resembling alcoholic liver disease that occurs in persons who consume little or no alcohol. NASH is a part of the spectrum of non-alcoholic fatty liver disease (NAFLD) and is defined by of the presence of ballooning (zone 3) hepatocellular injury and inflammation in addition to steatosis. NASH is typically associated with obesity, type II diabetes, dyslipidaemia and the metabolic syndrome. Fifty eligible patients of with NASH were included in the present clinical study. All the subjects were instructed regarding the study procedure and the monthly follow up visits and information regarding the contact person during emergency. All the patients were explained regarding the investigations that will be carried out during the period of the study. The subjects who have qualified the screening and willing to participate in the study were called for the study. They were instructed to take Liv.52 DS tablets 2 tablets twice daily for a period of 3 months. The predefined primary endpoints were improvement in steatohepatitis after the administration of Liv.52 DS Tablets. The efficacy parameters were improvements in clinical as well as liver function tests, ultrasonographic examination and a non-invasive NAFLD score which evaluates the severity of fatty liver fibrosis. The predefined secondary endpoints were incidences of adverse events and overall compliance to the drug therapy. There were no clinically significant adverse reactions; either reported or observed during the entire study period. The overall compliance to the treatment was good and no treatment discontinuations were reported.The results of the studies showed that Liv.52 DS is effective and safe in management of Non-Alcoholic Steatohepatitis.

Introduction

Fatty liver, also known as fatty liver disease is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis.

Steatosis is the process describing the abnormal retention of lipids within a cell. It reflects an impairment of the normal processes of synthesis and elimination of

triglyceride fat. Excess lipid accumulates in vesicles that displace the cytoplasm. When the vesicles are large enough to distort the nucleus, the condition is known as macrovesicular steatosis, otherwise the condition is known as microvesicular steatosis. Whilst not particularly detrimental to the cell in mild cases, large accumulations can disrupt cell constituents and functions.

Despite having multiple causes, fatty liver can be considered a single disease that occurs worldwide in those with excessive alcohol intake and those who are obese (with or without effects of insulin resistance). The condition is also associated with other diseases that influence fat metabolism[1] Morphologically it is difficult to distinguish alcoholic FLD from non-alcoholic FLD and both show micro-vesicular and macrovesicular fatty changes at different stages.

Accumulation of fat may also be accompanied by a progressive inflammation of the liver (hepatitis), called steatohepatitis. By considering the contribution by alcohol, fatty liver may be termed alcoholic steatosis or NASH.

Fatty liver is commonly associated with alcohol or metabolic syndrome (diabetes, hypertension, obesity and dyslipidaemia) but can also be due to any one of many causes[2, 3]

Metabolic: Abetalipoproteinemia, glycogen storage diseases, Weber-Christian disease, Wolman disease, acute fatty liver of pregnancy, lipodystrophy.

Nutritional: Malnutrition, total parenteral nutrition, severe weight loss, refeeding syndrome, jejuno-ileal bypass, gastric bypass, jejunal diverticulosis with bacterial overgrowth.

Drugs and toxins: Amiodarone, methotrexate, diltiazem, highly active antiretroviral therapy, glucocorticoids, tamoxifen, environmental hepatotoxins (e.g., phosphorus, mushroom poisoning).

Other: Inflammatory bowel disease, HIV, Hepatitis C especially genotype 3, and Alpha 1-antitrypsin deficiency[4].

Fatty change represents the intra-cytoplasmic accumulation of triglyceride (neutral fats). At the beginning, the hepatocytes present small fat vacuoles (liposomes) around the nucleus - microvesicular fatty change. In this stage liver cells are filled with multiple fat droplets that do not displace the centrally located nucleus. In the late stages, the size of the vacuoles increases pushing the nucleus to the periphery of the cell giving characteristic signet ring appearance - macrovesicular fatty change. These vesicles are well delineated and optically "empty" because fats dissolve during tissue processing. Large vacuoles may coalesce, producing fatty cysts - which are irreversible lesions. Macrovesicular steatosis is the most common form and is typically associated with alcohol, diabetes, obesity and corticosteroids. Acute fatty liver of pregnancy and Reye's syndrome are examples of severe liver disease caused by microvesicular fatty change. The diagnosis of steatosis is made when fat in the liver exceeds 5–10% by weight.[5, 6, 1]

Defects in fat metabolism are responsible for pathogenesis of NASH which may be due to imbalance in energy consumption and its combustion resulting in lipid storage or can be a consequence of peripheral resistance to insulin, whereby the transport of fatty acids from adipose tissue to the liver is increased[7,1].Impairment or inhibition of receptor molecules (PPAR-α, PPAR-γ and

SREBP1) that control the enzymes responsible for the oxidation and synthesis of fatty acids appears to contribute towards fat accumulation. In addition, alcoholism is known to damage mitochondria and other cellular structure further impairing cellular energy mechanism.

On the other hand non-alcoholic NASH may begin as excess of unmetabolised energy in liver cells. Hepatic steatosis is considered reversible and to some extent nonprogressive if there is cessation or removal of underlying cause.

Severe fatty liver is sometimes accompanied by inflammation, a situation that is referred to as steatohepatitis. Progression to alcoholic steatohepatitis (ASH) or non-alcoholic steatohepatitis (NASH) depend on persistence or severity of inciting cause. Pathological lesions in both conditions are similar. However, the extent of inflammatory response varies widely and does not always correlate with degree of fat accumulation. Steatosis (retention of lipid) and onset of steatohepatitis may represent successive stages in NASH progression[8].

Liver with extensive inflammation and high degree of steatosis often progresses to more severe forms of the disease[9]. Hepatocyte ballooning and hepatocyte necrosis of varying degree are often present at this stage. Liver cell death and inflammatory responses lead to the activation of stellate cells which play a pivotal role in hepatic fibrosis. The extent of fibrosis varies widely. Perisinusoidal fibrosis is most common, especially in adults, and predominates in zone 3 around the terminal hepatic veins.

The progression to cirrhosis may be influenced by the amount of fat and degree of steatohepatitis and by a variety of other sensitizing factors. In alcoholic FLD the transition to cirrhosis related to continued alcohol consumption is well documented but the process involved in non-alcoholic FLD is less clear.

There are no specific reliable treatment options available for NASH. During clinical studies with Liv.52 DS, in various liver disorders, some of the patients with NASH were benefited with Liv.52 DS. Therefore a clinical study was carried out to evaluate the safety and efficacy of Liv.52 DS in NASH. In this study anon-invasive reliable and widely accepted biomarker NAFLD score is adopted as an alternative to liver biopsy in assessing the severity of the fatty liver fibrosis, which serves as one of the parameter for the efficacy evaluation.

To evaluate the safety and efficacy of liv.52 DS in the management of Non-alcoholic Steatohepatitis (NASH)

Study design

The study was anopen clinical study conducted betweenDecember 2012 to November 2013, at Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India as per the ethical guidelines of Declaration of Helsinki. The study protocol, CRFs, regulatory clearance documents, product related information and informed consent form were submitted to the Institutional Ethics Committee and were approved by the same.

Materials and Methods

Inclusion criteria

Fifty subjects of either sex suffering from Steatohepatitis characterized by elevated liver enzymes and hepatomegaly with pain and discomfort in the right upper abdomen.

Exclusion criteria

Subjects with severe metabolic disorders, carcinoma of liver or pancreas, a 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, genetic and endocrinal disorders were excluded from the study. Subjects who had participated in a similar clinical investigation in the past four weeks, has used a similar product in the past four weeks and pregnant, and lactating women were excluded from the study

Study procedures

The demographic details of the subjects are mentioned in Table 1.Fifty eligible patients of with steatohepatitis were included in the study. All the subjects were instructed regarding the study procedure and the monthly follow up visits and information regarding the contact person during Emergency. All the patients were explained regarding the investigations that will be carried out during the period of the study.The subjects who have qualified the screening and willing to participate in the study were called for the study. They were instructed to take Liv.52 DS tablets 2 tablets twice daily for a period of 3 months

At the initial visit, a detailed medical history with special emphasis on family and past medical history was obtained from all subjects. In all subjects, a thorough systemic examination was done. All subjects were investigated by hematological and biochemical tests, which included Liver function tests, ultrasonography, and a non-invasive biomarker NAFLD scores for the severity of the liver fibrosis. Subjects were instructed to take Liv.52 DS tablets 2 tablets twice daily for a period of 3 months.

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*" (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).

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.

Follow-up and monitoring

Subjects were evaluated clinically on entry, at the end of 1st month, 2nd month and at the end of 3rd month. All the patients were followed for a period of 3 months and at each follow-up visit, the investigators recorded any information about either reported or observed adverse events.The score for the clinical assessment will be using a five-point scale on each visit: 5-Good improvement/ No complaints, 4-considerable improvement, 3- improvement, 2- no change, 1-worsening.

The scoring for ultrasonograhy was carriedout using a scale of 0-3, where 0-No fatty liver, 1- Mild fatty liver, 2 – moderate fatty liver, 3- Severe fatty liver in ultrasonography findings.

NAFLD score was evaluated with a score of NAFLD Score < -1.455 = F0-F2, NAFLD Score -1.455 to 0.675 = indeterminate score and NAFLD Score > 0.675 = F3-F4. NAFLD fibrosis score is calculated by a formula -1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet count (×109/L) - 0.66 × albumin. Overall clinical efficacy was assessed from a Six-point scale: Symptoms became worse = 6, No change = 5, Slight improvement = 4, Moderate improvement = 3, Marked improvement = 2 and Cured = 1

Primary and secondary end points

The predefined primary endpoint was improvement in liver functionparametresafter the administration of Liv.52 DS Tablets. The predefined secondary endpoints were incidences of adverse events (short- and long-term) and overall compliance to the drug therapy.

Statistical analysis

The values are expressed as Mean ± SD. Statistical analysis was performed by repeated measure of ANOVA followed by Friedman test withfollowed by Dunn's Multiple Comparison Test for between the group analyses followed by Paired t-test using Graph Pad Prism, Version 4.03 for windows, Graphpad Software, San Diego, California, USA. www.graphpad.com

Result and Discussion

Effect of Liv.52 DS on clinical parameters are shown in table 2.Significant improvement were observed in at entry for the values, the change in the values is considered for analyses at different intervals. Abdominal discomfort due to hepatomegaly which was 0.62±0.67initially improved to 2.38±0.67 at 1st month which further improved to 4.62±0.49 at 3rd month. Statistical analysis conducted within the group has shown that the level of significance was found to be $p<0.0001$ at 1st and 3rd monthas compared to initial value and at 3rd month as compared to Month 1 values. Fatigue which was 0.68±1.52 initially improved to 2.62±0.92 at 1st month which further improved to 4.62±0.49 at 3rd month with a significanceof $p<0.0001$ at 1st and 3rd month as compared to initial value and at 3rd month as compared to Month 1 values. Weakness was 0.44±0.58 initially improved to 2.54±0.54 at 1st month which further improved to 4.80±0.40 at 3rd month with a significance of $p<0.0001$ at 1st and 3rd month as compared to initial value and at 3rd month as compared to Month 1 values. Weight loss was 3.20±2.42 initially which improved to 3.96±1.44 at 1st month which further improved to 4.60±0.61 at 3rd month with a significance of $p<0.01$ at 3rd month as compared to initial value. Recurrent infections were4.32±1.17 initially which improved to 4.76±0.48 at 1st month which further improved to 5.00±0.00 at 3rd month. Statistical analysis conducted within the group has shown that the level of significance was found to be $p<0.05$ at 3rd month as compared to initial value.

The effect of Liv.52 DS on various blood parameters is shown in Table 3. Haemoglobin level which was 11.84 ±1.43 initially improved to 12.09 ± 2.11 at the end of the treatment. Total WBC count which was 8342.00±920.80 initially and8188.00±962.70 at the end of the treatment. Neutrophil count was 65.06±5.48 at entry and 65.52±6.19 at the end of

treatment. Similarly lymphocyte count showed improvement from 29.98±5.33 at entry and 29.76±6.06 at the end of the treatment. Monocyte count was 1.42±0.57 at entry and 1.32±0.59 at the end of treatment. ESR was 19.84±1.66 at entry which improved to 9.46±1.06 at the end of treatment with significance of $p<0.0478$. Platelets was 2.21±0.30 at entry and 2.24±0.29 at the end of treatment with significance of $p<0.0018$.

Effect of Liv.52 DS on various clinical chemistry parameters were evaluated as in Table 4. TSH levels was 4.56±18.68 initially which reduced to 3.73±18.83 at the end of the treatment with a significance of $p<0.0001$. Sugar Random levels was 105.30±17.02 initially which reduced to 56.89±53.24 at the end of the treatment with a significance of $p<0.0001$. Total Protein levels was 7.17±0.51 initially which reduced to 7.01±0.42 at the end of the treatment with a significance of p<0.0001. Bilirubin (Total) levels was 0.70±0.21 initially which reduced to 0.64±0.19 at the end of the treatment with a significance of $p<0.0004$. Alkaline phosphatase levels was 214.80±55.08 initially which reduced to 198.80±52.65 at the end of the treatment with a significance of $p<0.0001$. SGOT levels was 32.08±4.62 initially which reduced to 29.25±4.32 at the end of the treatment with a significance of $p<0.0001$. SGPT levels showed reduction from 61.12±10.51 to 41.08±7.69 after treatment with a significance of p<0.0001.Cholesterol Total levels showed reduction from 204.30±18.23 to 181.30±15.26 after treatment with a significance of p<0.0001. Serum Triglyceride levels showed reduction from 279.40±41.09 to 191.40±21.28 after treatment with a significance of $p<0.0001$.

Effect of Liv.52 as evaluated by Ultrasonography is shown in table 5, fig1. The score before treatment was 1.58±0.64 which improved to 0.66±0.56 with a significance of $p<0.0001$.

The effect of Liv.52 DS by the NAFLD score is provided in Table 6. NAFLD score which was -0.98±0.39 at entry improved to -1.22±0.41 which signifies that the liver fibrosis became less severe as measured by the non-invasive tool.

The overall Impression of Liv.52 DS tablet is shown in Table7. The investigators opined that2% of the subjects had marked improvement. 20% of the subjects had moderate improvement; slight improvements were seen in 36% of subjects, meaning 58% of the subjects showed varying response to the therapy.Whereas 38% subjects showed no changes and in 4% of subject symptoms got worsen.

Liv.52 DS Tablet is a hepatospecific formulation, designed for the management of liver disorders. It has a wide spectrum of therapeutic applications. It restores the metabolic efficiency of the liver in various etiological forms of hepatocellular jaundice like infective and chronic active hepatitis, drug-induced hepatitis and alcohol-induced hepatic damage. It increases appetite. It corrects the hepatitis, and cirrhotic conditions, and in any hepatotoxic drug regimen. It is a supportive treatment during hemodialysis, and a useful adjuvant with hepatotoxic drugs (e.g., statins).

Eight active medicinal herbs viz., *Capparis spinosa, Cichoriumintybus, Mandura bhasma, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achilleamille foliuma* nd *Tamarix gallica* were carefully selected during the product development. These herbs possess significant hepatoprotective activity and have been used for centuries as a part of the Ayurvedic approach to healthcare.

Capparisspinosa

P-Methoxy benzoic acid from *Cappariss pinosa* has potent hepato protective activity against chemically-induced hepatotoxicity, prevents elevation of malondialdehyde levels (plasma and hepatic) and enzyme levels (AST and ALT)[10-12]. It improves the functional efficiency of the liver and spleen, with protective action on the histological architecture of the liver, and a salutary effect on liver glycogen and serum proteins[13]. Flavonoids of *Capparisspinosa* have significant antioxidant activity, as demonstrated by lipid peroxidation, bleaching of free radicals, and auto-oxidation of iron ions[14].

Cichoriumintybus

Cichoriumintybus protects the liver against alcohol toxicity. It increases circulating leukocytes, splenic plaque-forming cells, hemagglutination titers, secondary IgG antibody response, phagocytic activity, natural killer cell activity, cell proliferation, and interferon gamma secretion[15,16]. Its hepatoprotective activity suppresses the oxidative degradation of DNA in tissue debris[17]. It also has potent antioxidant action, as evident by its free radical scavenging effects, inhibition of hydrogen peroxide and iron chelation[18,19].

Solanum nigrum

Solanum nigrum protects DNA against oxidative damage[20], and also acts as a potent scavenger of hydroxyl and diphenylpicrylhydrazyl radicals[21]. The cytoprotective effect of *Solanum nigrum* against gentamicin-induced toxicity showed a significant inhibition of cytotoxicity, and hydroxyl radical scavenging potential[22].

Terminalia arjuna

Terminalia arjuna reduces cholesterol levels and is also useful in liver disorders[23,24].Ithas potent antioxidant activity, which is due to its effects on lipid peroxidation[25]. Arjunaphthanoloside from *Terminalia arjuna* inhibits nitric oxide production,and terminoside A decreases inducible nitric oxide synthase levels in lipopolysaccharide-stimulated peritoneal macrophages[26]. It has strong antiviral activity, inhibiting viral attachment and penetration[27]. It also has supportive antibacterial activity[28].

Cassia occidentalis

Cassia occidentalis has significant hepato protective effects in chemically-induced liver damage[29]. It modulates hepatic enzymes, which provides hepato protection[30].

Achilleamille folium

*Achilleamille folium*is beneficial in chronic hepatitis[31] and has anti-hepatoma activity[32].

Tamarixgallica

Tamarixgallica is a hepatic stimulant, digestive and hepatoprotective, and has a salutary effect on liver glycogen and serum proteins[33].

Mandura bhasma

Mandura bhasma has hepatoprotective property, and is beneficial in chemically-induced hepatotoxicity as it prevents changes in liver enzyme activities[34]. Mandurabhasma is a powerful hematinic and tonic[35].

Table.1 Demographic Details at baseline(n=50)		
Age in years		48.28 ± 9.92
Gender	Male	21
	Female	29
Diet	Veg	13
	Non-Veg	36

Table.2 Effect of Liv.52 DS on clinical parameters			
Parameters	**Screening**	**1st month**	**3rd Month**
Abdominal discomfort due to hepatomegaly	0.62±0.67	2.38±0.67*a	4.62±0.49*a,b
Fatigue	0.68±1.52	2.62±0.92*a	4.74±0.44*a,b
Weakness	0.44±0.58	2.54±0.54*a	4.80±0.40*a,b
Weight loss	3.20±2.42	3.96±1.44	4.60±0.61*c
Recurrent infections	4.32±1.17	4.76±0.48	5.00±0.00*d
Statistical test: Friedman test with followed by Dunn's Multiple Comparison Test. a= $p<0.001$ As compared to at entry values b= $p<0.001$ As compared to 1st month values c= $p<0.01$ As compared to at entry values d= $p<0.05$ As compared to at entry values			

Table.3 Haematology and Biochemical Investigations			
	Intial	**After**	**Significance**
Haemoglobin (g/dl)	11.84 ±1.43	12.09±2.11	NS
WBC (cells /cumm)	8342.00±920.80	8188.00±962.70	NS
Neutrophils %	65.06±5.48	65.52±6.19	NS
Lymphocytes %	29.98±5.33	29.76±6.06	NS
Eosinophils %	3.54±0.79	3.40±0.81	NS
Monocytes %	1.42±0.57	1.32±0.59	NS
Basophils %	0.00±0.00	0.00±0.00	NS
ESR mm/hr	9.84±1.66	9.46±1.06	$p<0.0478$
Platelets (lakh cells/ml)	2.21±0.30	2.24±0.29	$p<0.0018$
Statistical test: Paired “t” test NS:Not Significant			

Table.4 Clinical Chemistry Parameters (Mean±SD)		
Particulars	**Intial**	**After**
Thyroid stimulating Hormone (mu/l)	4.56±18.68	3.73±18.83*
Random Blood Sugar (RBS)	105.30±17.02	56.89±53.24*
Total protein (gm/dl)	7.17±0.51	7.01±0.42*
Bilirubin (Total)	0.70±0.21	0.64±0.19**
Alkaline phosphatase (U/l)	214.80±55.08	198.80±52.65*
SGOT (AST) (U/l)	32.08±4.62	29.25±4.32*
SGPT (ALT)(U/l)	61.12±10.51	41.08±7.69*
Total Cholesterol (mg/dl)	204.30±18.23	181.30±15.26*
Serum Triglyceride (mg/dl)	279.40±41.09	191.40±21.28*
Statistical test: Paired "t" test *=p<0.0001; **=p<0.0004		

Table.5 Effect of Liv.52 as evaluated by Ultrasonography(Mean±SD)		
Before treatment	After treatment	Significance
1.58±0.64	0.66±0.56*	*p<0.0001

Fig.1 Ultrasonographic Findings with Liv.52 DS

Fig 1a: Hepatomegaly with Grade 1 fatty infiltration hepatomegaly and fatty infiltration (Pre-treatment)

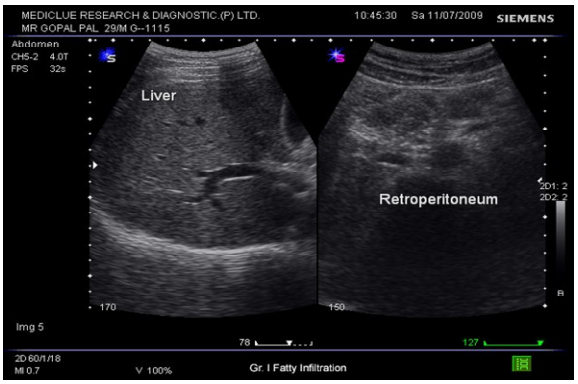

Fig 1b: Marked improvement in (Post-treatment with Liv.52 DS)

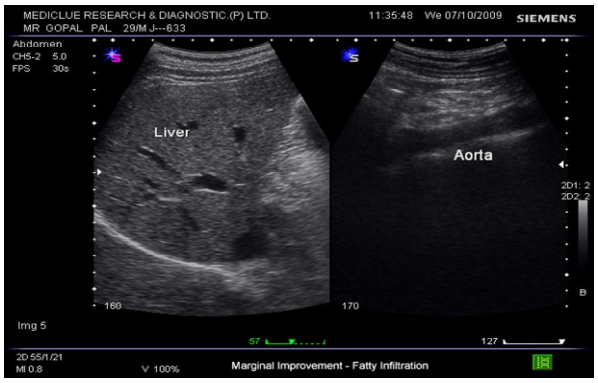

Table.6 Effect of Liv.52 DS on NAFLD score	
At entry	**End of the study**
-0.98±0.39	-1.22±0.41
NAFLD score interpretation: NAFLD Score < -1.455 = F0-F2 NAFLD Score -1.455 to 0.675 = Indeterminate score NAFLD Score > 0.675 = F3-F4.	

Table.7 Overall Impression*		
Scoring for treatment response	**Number**	**Percentage (%)**
Symptoms became worse = 6	2	4
No change = 5	19	38
Slight improvement = 4	18	36
Moderate improvement = 3	10	20
Marked improvement = 2	1	2
Cured = 1	0	0
Total	50	100
*58% of the patients showed varying degree of improvements		

The efficacy of the formulation could be attributed to the synergistic activity of the herbs in the formulation

Liver biopsy is considered the gold standard for the diagnosis and assessment of fibrosis severity but has several limitations, such as sampling variability, invasiveness and expense. In this study a non-invasive biomarker to assess the severity of liver fibrosis which is an alternate to liver biopsy was used. Patients with NASH can have a significant progression of fibrosis within a few years. Recently, a simple, noninvasive tool used for liver fibrosis assessment has been developed. This new scoring system, the NAFLD fibrosis score (NFS), is a composite score of age, hyperglycaemia, body mass index, platelet count, albumin, and aspartate aminotransferase and alanine aminotransferase (AST/ALT) ratio. NAFLD fibrosis score = -1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet count (×109/L) - 0.66 × albumin.[36]

Conclusion

The clinical study clearly shows that Liv.52 DS is beneficial in improving clinical and liverfunction parameters as well as in Ultrasonographic and NAFLD scores of NASH.There were no adverse reactions either observed or reported during the study period. Therefore, it may be concluded that Liv.52 DS Tablet is efficient in management of Steatohepatitis and safe for usage in Liver diseases.

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