Medicine Update (2004): 12(2), 51-61

Meta-analysis of 50 Phase III clinical trials in evaluation of efficacy and safety of Liv.52 in infective hepatitis

Kolhapure, **S.A.,** *M.D.,* Senior Medical Advisor,
and
Mitra, S.K., *M.D.,* Executive Director,Research and Technical Service,
R&D Center, The Himalaya Drug Company, Bangalore, India

ABSTRACT

Hepatitis A (HA) has a worldwide distribution occurring in epidemic and sporadic patterns. Hepatitis A is an acute, but benign form of viral hepatitis and early renormalizations of hepatic functions with symptomatic and clinical recovery are the objectives in the clinical management of HA. meta-analysis is the term used to describe quantitative methods for combining information across different studies and this study was planned for meta-analysis of the efficacy and safety of Liv.52 tablet and syrup, in HA, as reported in 50 published study reports.

All study reports evaluating efficacy and safety of Liv.52, were included for the meta-analysis, regardless of the study design, but Phase I and II clinical, experimental and preclinical studies were excluded from the meta-analysis. Each study was abstracted for the number and ages of enrolled patients, changes in the biochemical parameters [serum bilirubin (SB), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SAP), serum albumin (SA) and serum globulin (SG), and prothrombin time (PT)] from baseline to values at the end of study and total duration of clinical recovery were recorded. Incidence of adverse events during the study period and patient compliance to the drug treatment was noted. The predefined primary endpoints were to determine level of statistical significance for symptomatic improvement, renormalization of biochemical parameters and total duration of clinical recovery. The predefined secondary endpoints were incidence of adverse events during the study period and compliance to the drug treatment.

Total 50 clinical studies conducted over a span of 30 years were considered for this meta-analysis and the mean duration of these studies was 6.62 months. Out of total fifty studies, 3 were double-blind placebo-controlled studies, 21 were placebo-controlled studies, 22 were non-comparative studies and 4 studies were case reports. Total 4490 patients were enrolled in these studies and 233 children were part of study population. Cumulative data analysis showed a significant reduction in the mean SB, SGOT, SGPT, AP levels, PT and mean period required for total (symptomatic, clinical and biochemical) recovery. The decreased SA and SG levels were also increased significantly, when compared to the pre-treatment values, in all studies. There were no reported or observed significant adverse events in all trials and the overall drug compliance was excellent. Therefore, this metaanalysis concludes that, Liv.52 tablets and syrup are effective and safe in the management of hepatitis.

INTRODUCTION

Meta-analysis (also referred as 'quantitative synthesis' or 'overview analysis') is the term used to describe quantitative methods for combining information across different studies. Glass coined the term `meta-analysis' in 1976[1], to describe this idea of utilizing information in many studies of the same effect, although the concept itself is much older (dating back to the 1930s studies by Fisher and Pearson). Today, the term `meta-analysis' is reserved for a

situation where one combines numerical effects from a collection of studies, rather than giving a more general non-quantitative overview.

The term “Hepatitis A (HA)” has replaced all previous designations like ‘type A viral hepatitis’, ‘infectious hepatitis’, ‘epidemic hepatitis’, ‘epidemic jaundice’, ‘catarrhal jaundice’, ‘infectious icterus’, ‘Botkin’s disease’, and ‘MS-1 hepatitis’. HA has a worldwide distribution and occurs in both epidemic and sporadic patterns. In developing countries, the incidence of HA in adults is relatively low due to the exposure to the virus in childhood and most adults demonstrate an immunity that provides lifelong protection against reinfection[2].

Hepatitis A is an acute but benign form of viral hepatitis caused by an RNA virus that does not persist in the blood serum. Hepatitis A virus (HAV) is of the enterovirus group of the Picornaviridae family and HAV has a single molecule of RNA surrounded by a small (27 nm diameter) protein capsid. HA is a food/waterborne disease, with feco-oral transmission and the incubation period is variable (from 15 to 25 days). Incubation period is inversely proportional to infective dose and the infectious dose presumably is 10-100 virus particles[2].

Hepatitis A is usually a mild illness characterized by sudden onset of fever, malaise, nausea, anorexia and abdominal discomfort, followed after 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 the incubation period, indicating that transmission usually occurs before or around the onset of jaundice [2].

Hepatitis A is diagnosed by finding IgM anti-HAV in serum during the acute or early convalescent phases of disease. A moderate increase in conjugated bilirubin, with marked elevation of SGOT, SGPT and Lactate Dehydrogenase isoenzymes (LDH) are noted in HA (low Na+ and Cl- levels may be seen due to severe vomiting). Marginal elevation of alkaline phosphatase is seen due to compression of intrahepatic biliary canaliculi by swollen parenchymatous cells. Triglycerides and cholesterol may be temporarily and marginally lowered[2].

Hepatitis A is the least serious of the hepatitis viruses, as it does not kill liver cells and also, there is no risk for a chronic form. Hepatitis A is a self-limiting disease and no specific treatment is available. 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 transmission of infection to others. Therefore, early renormalizations of hepatic functions with symptomatic and clinical recovery are the objectives in the clinical management of HA.

Liv.52 tablet and syrup are polyherbal formulations, used extensively in the management of HA. Liv.52 syrup contains powders of *Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium* and *Tamarix gallica* (in Liv.52 tablet, Mandur bhasma is added)**.** This study was planned for meta-analysis of the efficacy and safety of Liv.52 tablet and syrup, in HA, as reported in 50 published study reports.

AIM OF STUDY

The aim of the study was to meta-analyze the efficacy and short- and long-term safety of Liv.52 in HA, as reported in fifty published study reports.

STUDY DESIGN

This study was a cumulative meta-analysis of fifty published study reports of Liv.52 in HA.

MATERIALS AND METHODS

Inclusion criteria

All published study reports evaluating efficacy and safety of Liv.52 were included for the meta-analysis, regardless of the study design (either double-blind placebo-controlled studies, placebo-controlled studies, open, non-comparative studies, or case reports with less than ten patients).

Exclusion criteria

Phases I and II clinical, experimental and preclinical studies were excluded from the meta-analysis.

Study procedures

All studies were categorized into four subgroups as per the design of the study. Group I included all double-blind placebo-controlled studies, group II included placebo-controlled studies, group III included all open (non-comparative) studies and group IV included case reports, in which less than 10 patients were enrolled.

Each study was abstracted with emphasis on the number and ages of enrolled patients. Changes in the biochemical parameters (serum bilirubin (SB), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SAP), serum albumin (SA), serum globulin (SG), and prothrombin time (PT)) from baseline to values at the end of study, and total duration of clinical recovery were recorded. Incidence of adverse events during the study period and patient compliance to the drug treatment was noted.

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 study medication was predefined as *"Unrelated"* (a reaction that does not follow a reasonable temporal sequence from the 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 endpoints were to determine level of statistical significance for the following parameters: symptomatic improvement, renormalization of biochemical parameters and total duration of clinical recovery. The predefined secondary endpoints were incidence of adverse events during the study period and compliance to the drug treatment.

Statistical analysis

Statistical analysis was done according to intention-to-treat principles. The data was evaluated for normality by the Shapiro-Wilk normality test, while Gaussian approximation and status of exactness was evaluated by Wilcoxon Signed-Rank Test. 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

Total fifty clinical studies that evaluated the efficacy and safety of Liv.52 in HA, conducted over a span of 30 years (from 1967 to 1997) were considered for this meta-analysis. The mean duration of these studies was 6.62 months and the total study duration of all trials was 331 months (minimum period=1.0 month, maximum period=36.00 months, mean of means (M)=6.62, Std. deviation (SD)=6.645, Std. error of mean (SEM)=0.9398, lower 99% confidence interval (CI) of mean=4.097, upper 99% CI of mean=9.143, W=0.6497 (Shapiro-Wilk normality test), $p<0.0001$, significant (S)) (Table 1). Out of fifty studies, three were double-blind placebo-controlled studies, twenty-one were placebo-controlled studies, twenty-two were open (non-comparative)

Figure 1: Distribution of studies as per study design

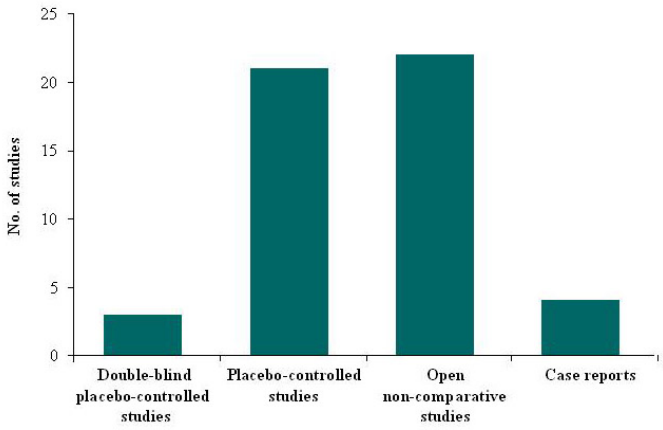


Figure 2: Distribution of patients as per received treatment

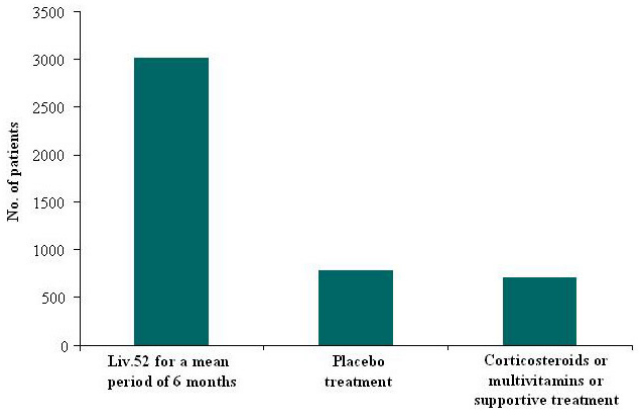
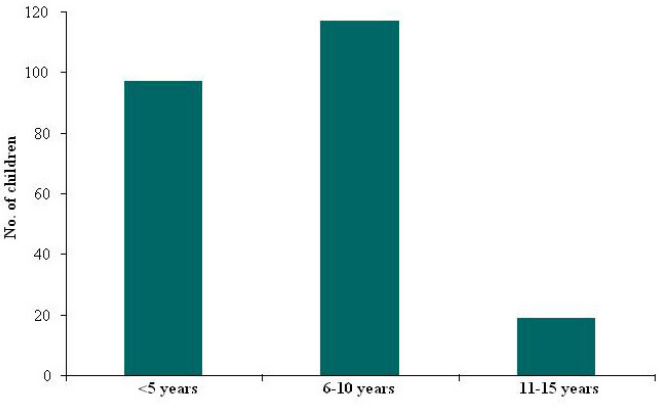


Figure 3: Agewise distribution of children enrolled in various trials



studies and four studies were case reports with less than ten enrolled patients (Figure 1).

Total 4490 patients were enrolled in these studies and 3007 patients received Liv.52 for a mean period of six months. From the control group, 785 patients consumed placebo and other patients consumed corticosteroids, multivitamins, or other treatment (Figure 2).

Two hundred and thirty-three children were part of a study population classified as per their age: 97 children below age of 5 years, 117 children between of 6 to 10 years and 19 children between 11 to 15 years (Figure 3).

The mean serum bilirubin values were elevated at the time of the enrolment, in all patients in these studies. Cumulative data analysis showed a significant reduction in the mean serum bilirubin level, at the end of all studies (parameter, value before treatment, value after treatment: minimum=2.660 and 0.5000, maximum=12.80 and 8.050, mean of means =7.030 and 2.014, SD=2.965 and 1.840, SEM=0.6630 and 0.4115, lower 95% CI of mean=5.642 and 1.153, upper 95% CI of mean=8.418 and 2.875, $p<0.0001$ and $p<0.0001$ (Gaussian approximation by Wilcoxon Signed-Rank Test), S; and $t=8.255$, $p<0.0001$, S, mean of differences = 5.016, 95% confidence interval =3.744 to 6.288 (paired t test) (Figure 4).

Similarly, the elevated serum glutamic oxaloacetic transaminase (SGOT) levels were reduced significantly, when compared to the pre-treatment values, in all the above studies. (minimum=24.00 and 0.0, maximum=140.0 and 39.40, mean of means=78.37 and 21.60, SD=37.47 and 13.47,

Figure 4: Mean of means of bilirubin in Hepatitis A patients before and after treatment byLiv.52

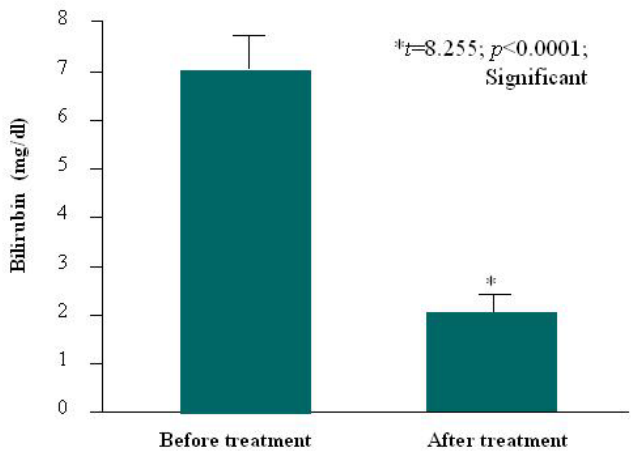


Figure 5: Mean of means of SGOT in Hepatitis A patients before and after treatment by Liv.52

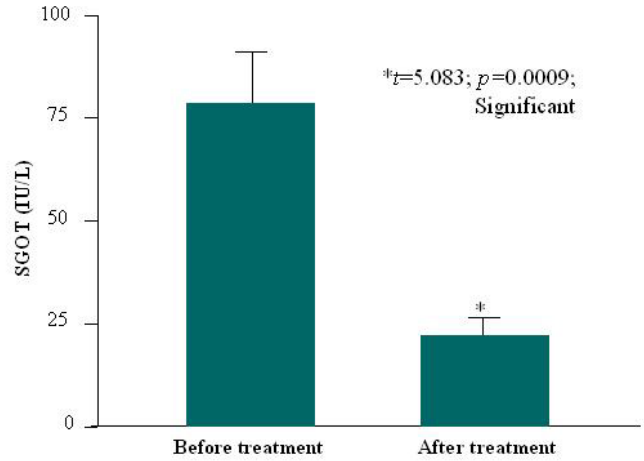
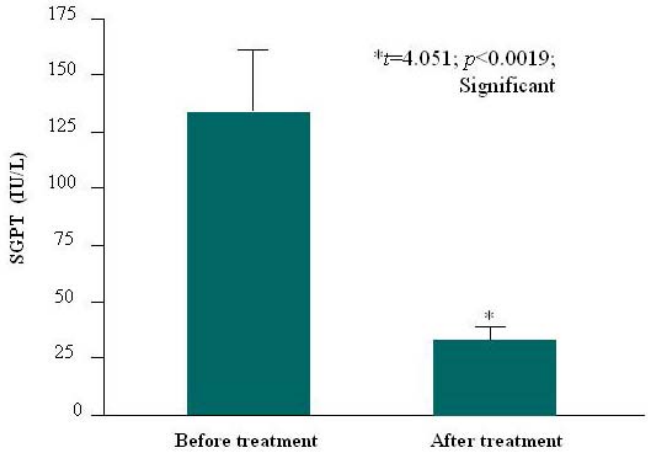
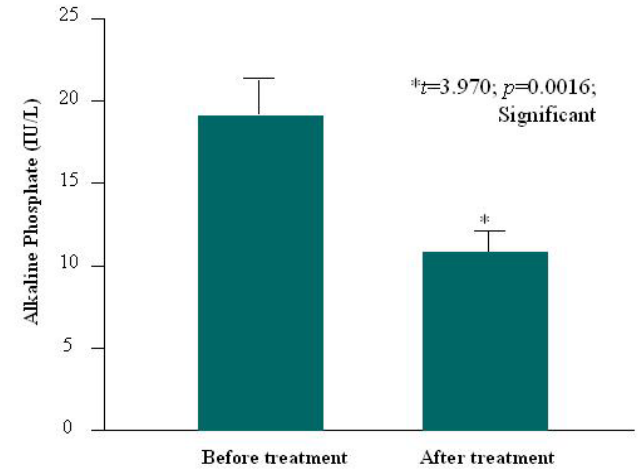


Figure 6: Mean of means SGPT of Hepatitis A patients before and after treatment by Liv.52



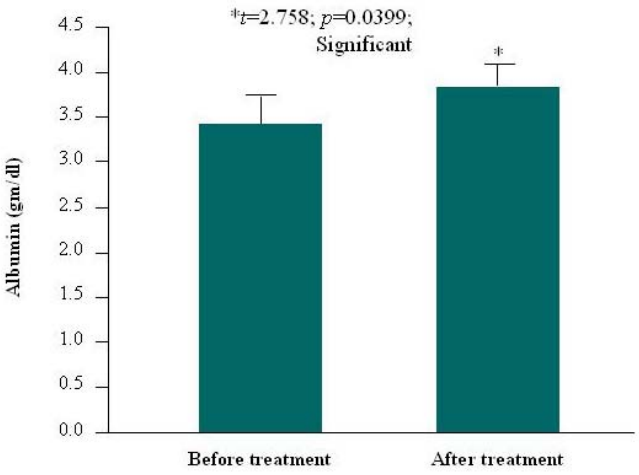
SEM=12.49 and 4.488, lower 99% CI of mean=36.47 and 6.539, upper 99% CI of mean=120.3 and 36.66, p=0.0020 and p=0.0039 (Wilcoxon Signed-Rank Test for exactness), S; and t=5.083, p=0.0009, mean of differences=56.78, 95% confidence interval=31.02 to 82.53 (Paired 't' test) (Figure 5).

Figure 7: Mean of means of alkaline phosphatase in Hepatitis A patients before and after treatment by Liv.52



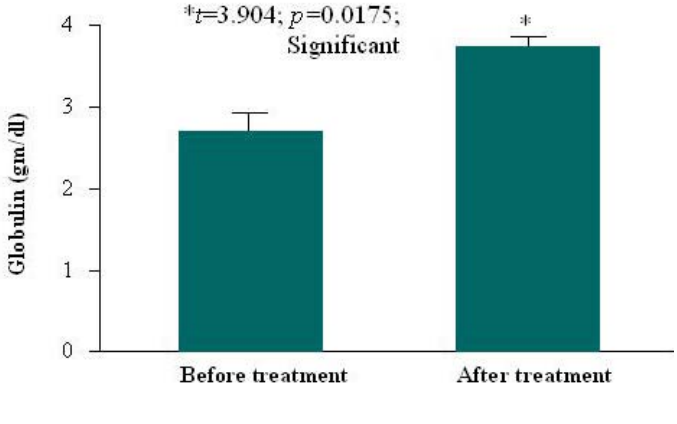
The elevated serum glutamic pyruvic transaminase (SGPT) levels were also reduced significantly, when compared to the pre-treatment values, in all of the above studies (minimum=30.00 and 0.0, maximum=344.0 and 91.59, mean of means =133.4 and 33.59, SD=94.22 and 23.48, SEM=27.20 and 6.779, lower 95% CI of mean=73.55and 18.67, upper 95% CI of mean=193.3 and 48.51, p=0.0002 and p=0.0005 (Wilcoxon Signed-Rank Test for exactness), S; and t=4.051, p=0.0019, mean of differences=99.84, 95% confidence interval=45.59 to 154.1(Paired 't' test) (Figure 6).

Figure 8: Mean of means of albumin in Hepatitis A patients before and after treatment by Liv.52



The elevated alkaline phosphatase levels were also reduced significantly, when compared to the pre-treatment values, in all of the above studies (Minimum= 9.540 and 0.0, maximum=32.96 and 16.70, mean of means=19.09 and 10.72, SD=7.761 and 4.722, SEM=2.074 and 1.262, lower 95% CI of mean=14.61 and 7.996, upper 95% CI of mean=23.57 and 13.45, p<0.0001and p<0.0001 (Wilcoxon Signed-Rank Test for exactness), S; and t=3.970, p=0.0016, mean of differences=8.369, 95% confidence interval=3.816 to 12.92 (Paired 't' test) (Figure 7).

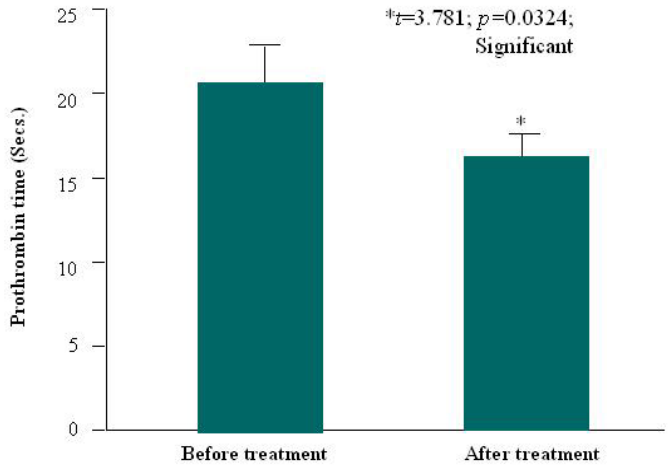
Figure 9: Mean of means of serum globulin in Hepatitis A patients before and after treatment by Liv.52



Decreased serum albumin levels also increased significantly compared to pre-treatment values in all studies (minimum=2.100 and 3.010, maximum=4.300 and 4.500, mean of means=3.405 and 3.843, SD=0.7928 and 0.6042, SEM=0.3237 and

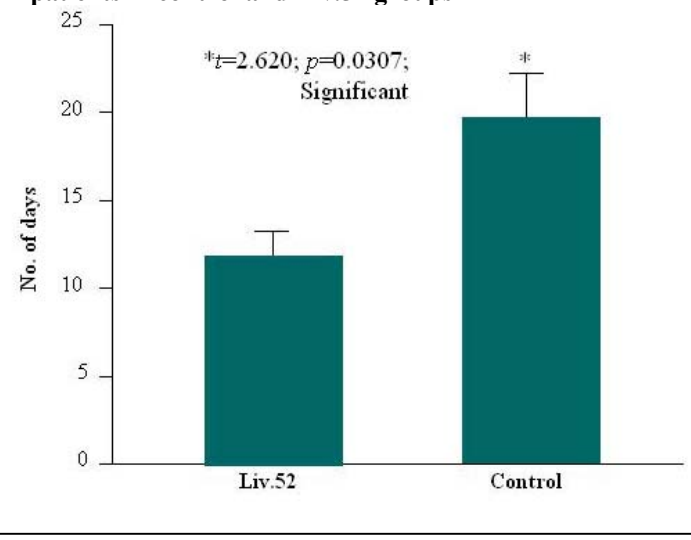
0.2467, lower 95% CI of mean=2.573 and 3.209, upper 95% CI of mean=4.237 and 4.477, p=0.0156 and p=0.0156 (Wilcoxon Signed-Rank Test for exactness), S; and t=2.758, p=0.0399, mean of differences= 0.4383, 95% confidence interval = 0.8469 to 0.02975 (Paired 't' test) (Figure 8).

Figure 10: Mean of means of prothrombin time in Hepatitis A patients before and after treatment by Liv.52



Similarly, the decreased serum globulin levels were also increased significantly, when compared to the pre-treatment values, in all of the above studies. (minimum=2.300 and 3.580, maximum=3.160 and 4.100, mean of means=2.662 and 3.696, SD=0.4519 and 0.2260, SEM=0.2021 and 0.1011, lower 95% CI of mean=2.101 and 3.415, upper 95% CI of mean=3.223 and 3.977, p=0.0031 and p=0.0031 (Wilcoxon Signed-Rank Test for exactness), S; and t=3.904, p=0.0175, mean of differences=1.034, 95% confidence interval=0.2987 to 1.769 (Paired 't' test) (Figure 9).

Figure 11: Mean of means of period for recovery in Hepatitis A patients in control and Liv.52 groups



Raised prothrombin time levels also reduced significantly compared to pre-treatment values in all studies (minimum=17.00 and 15.00, maximum=26.00 and 20.00, mean of means=20.75 and 16.25, SD=4.113 and 2.500, SEM=2.056 and 1.250, lower 95% CI of mean=14.21 and 12.27, upper 95% CI of mean=27.29 and 20.23, p=0.0062 and p=0.0062 (Wilcoxon Signed-Rank Test for exactness), S; and t=3.781, p=0.0324, mean of differences=4.500, 95% confidence interval=0.7127 to 8.287 (Paired 't' test) (Figure 10).

There was highly significant reduction in the mean period required for total (symptomatic, clinical and biochemical) recovery, as compared to placebo (minimum=9.000 and 14.00, maximum=17.00 and 26.60, mean of means=11.84 and 19.56, SD=3.189 and 5.764, SEM=1.426 and 2.578, lower 95% CI of mean=7.882 and 12.40, upper 95% CI of mean=15.80 and 26.72, p=0.0031 and p=0.0031 (Wilcoxon Signed-Rank Test for exactness), S; and t=2.620, p=0.0307, difference between means = 7.718 ± 2.946, 95% confidence interval=14.51 to 0.9241 (Paired 't' test) (Figure 11).

There were no reported or observed significant adverse events in any trial and the overall drug compliance was excellent.

DISCUSSION

The number of papers published on meta-analysis in medical research has increased sharply in the past decade, however the merits and perils of the meta-analysis continue to be debated in the medical community[53,54]. A useful definition of meta-analysis was given by Huque as: "A statistical analysis that combines or integrates the results of several independent clinical trials considered by the analyst to be 'combinable'".[55] The terminology, however, is still debated, and expressions used concurrently include "overview," "pooling," and "quantitative synthesis." The present consensus is that the term meta-analysis should be used to describe the statistical integration of separate studies, whereas "systematic review" is appropriate for denoting any review of a body of data that uses clearly defined methods and criteria. Systematic reviews can include meta-analyses, appraisals of single trials, and other sources of evidence[56]. "Meta-analysis" has recently been included as a Medical Subject Heading (MeSH) and publication type within the Medline indexing system of the National Library of Medicine[57].

A single study often cannot detect or exclude with certainty clinically relevant differences in the effects of two treatments. A trial may thus show no significant treatment effect when in reality such an effect exists (false negative result - type II error). Generally better recognized is the type I error (significant difference due to chance and the probability that corresponds to the *p* value)[58]. Cumulative meta-analysis is defined as the repeated

Table 1: Details of included trials for meta-analysis

Sl. No.	Name	Year	Trial design	Duration (months)	No. of patients
1	Sule *et al.*[3]	1968	C	36	150
2	Jaffari *et al.*[4]	1969	C	1	48
3	Arora[5]	1969	C	6	626
4	Doddagoudar *et al.*[6]	1970	OP	4	110
5	Lala Prasad *et al.*[7]	1971	CR	3	7
6	Mukherjee *et al.*[8]	1971	C	5	25
7	Deshpande *et al.*[9]	1971	OP	6	100
8	Dasgupta *et al.*[10]	1971	OP	24	10
9	Patel *et al.*[11]	1972	C	4	52
10	Gupta *et al.*[12]	1972	CR	4	2
11	Ramalingam *et al.*[13]	1972	C	3	250
12	Dave *et al.*[14]	1972	C	4	35
13	Gupta *et al.*[15]	1972	OP	5	36
14	Sarkar[16]	1973	C	2	45
15	Shishupal Ram *et al.*[17]	1974	OP	4	36
16	Krishnamurthy Leela *et al.*[18]	1974	DB	6	60
17	Reddi *et al.*[19]	1974	DB	24	60
18	Jain *et al.*[20]	1974	C	18	27
19	Sudhakar Rao *et al.*[21]	1974	C	4	120
20	Khetrapal *et al.*[22]	1974	C	6	49
21	Sharma *et al.*[23]	1974	OP	2	58
22	Deshpande[24]	1974	OP	12	289
23	Sinha[25]	1974	OP	2	222
24	Bhandari *et al.*[26]	1974	C	4	48
25	Mazumdar *et al.*[27]	1974	C	4	100
26	Mitra *et al.*[28]	1974	OP	18	84
27	Rath *et al.*[29]	1975	OP	3	15
28	Agarwal *et al.*[30]	1976	C	6	125
29	Chavan *et al.*[31]	1976	OP	4	130
30	Sama *et al.*[32]	1976	C	8	34
31	Singh *et al.*[33]	1976	C	12	16
32	Bannerjee *et al.*[34]	1977	OP	10	53
33	Singh *et al.*[35]	1977	OP	8	50
34	Habibullah *et al.*[36]	1978	DB	6	50
35	Mehrotra *et al.*[37]	1978	C	4	50
36	Sethi *et al.*[38]	1978	CR	6	16
37	Ila V. Desai *et al.*[39]	1978	C	5	130
38	Biswas *et al.*[40]	1980	OP	2	70
39	Mandal *et al.*[41]	1980	CR	3	1
40	Saxena *et al.*[42]	1980	C	4	30
41	Kesarkar *et al.*[43]	1981	OP	3	225
42	Chowdhary *et al.*[44]	1981	OP	4	160
43	Mishra *et al.*[45]	1981	OP	8	54
44	Mandal *et al.*[46]	1983	C	2	104
45	Sreenivas Rao[47]	1983	OP	2	65
46	Bharadia *et al.*[48]	1986	OP	3	53
47	Vijaykumr *et al.*[49]	1988	OP	5	32
48	Patney *et al.*[50]	1988	C	4	325
49	Dange *et al.*[51]	1989	OP	2	34
50	Kalab *et al.*[52]	1997	OP	6	19

C: placebo-controlled; DB: Double-blind placebo-controlled; OP: Open-non-comparative; CR: Case reports

performance of meta-analysis whenever a new trial becomes available for inclusion. Such cumulative meta-analysis can retrospectively identify the point in time when a treatment effect first reached conventional levels of significance[59].

Meta-analysis thus not only consists of the combination of data but also includes the epidemiological exploration and evaluation of results ("epidemiology of results")[60]. Therefore, new hypotheses that were not posed in single studies can be tested in meta-analyses[61]. The number of patients included in clinical trials is often inadequate as in some cases the required sample size may be difficult to achieve[62]. Meta-analysis can, nevertheless, lead to the identification of the most promising or urgent research question, and may permit a more accurate calculation of the sample sizes needed in future studies[63]. The goals of meta-analysis are to enable the overall significance of an effect to be evaluated, based on the multiple studies available; to estimate an overall effect size by combining the individual estimates in multiple studies. The problem of comparability of different study designs and effects of a difference in quality in multiple studies is still not resolved and there are no guidelines for the same[64].

While evaluating the efficacy of Liv.52 in HA, the primary endpoints (quantitative comparison of pooled data for biochemical parameters by 'before' and 'after' tests) were selected by their specific significance in natural history of HA. Most adult-acquired liver disease causes impairment in bilirubin secretion from the liver, which causes elevation of SB in the blood. In acute liver disease, SB is usually elevated relative to the severity of the acute process. Blood levels of SGOT (also referred as aspartate aminotransferase (AST)) and SGPT (also referred as Alanine aminotransferase (ALT)) are elevated in all types of hepatitis (viral, alcoholic, drug-induced, etc.). An elevation in the level of SAP suggests disease of the bile ducts (bile duct obstruction, primary biliary cirrhosis or primary sclerosing cholangitis). Albumin is the major protein synthesized in the liver, which circulates in the bloodstream and low SA concentrations indicate poor liver function. The PT is prolonged when the blood concentrations of some of the clotting factors made by the liver are low and in acute liver diseases, the PT can be prolonged.

This meta-analysis included data of 50 clinical studies (24 placebo-controlled studies and 26 open non-comparative studies) conducted over a 30 years, in 4490 patients (including 117 children) and this sample size is large enough for calculating the intervention (drug) effect. The recommended method for calculating sample size is by the formula: $n=4pq/L^2$ (p=prevalence, q=100 – p, L=1.96) and by this formula, this sample size is large enough for a disease, with a very high prevalence. There was significant symptomatic control observed in a week's time. The cumulative analysis revealed significant reduction in the levels of mean SB, SGOT, SGPT and SAP and there was renormalization of protein levels and PT. There was significant reduction in the mean period required for total recovery, as compared to placebo. There were no reported or observed significant adverse events in all trials and the overall patient compliance to the treatment was excellent. Therefore, as per the predefined primary and secondary endpoints the efficacy and safety of Liv.52 in HA is clinically, biochemically and statistically well proved. These significant effects might be due to the synergistic properties and actions of the ingredients of Liv.52.

Khanfar *et al*. isolated and identified active ingredients of *Capparis spinosa* as beta-sitosterylglucoside-6'-octadecanoate and 3-methyl-2-butenyl-beta-glucoside[65]**.** p-Methoxy benzoic acid isolated from *Capparis spinosa* was found to possess potent hepatoprotective activity against CCl_4, paracetamol (*in vivo*) and in thioacetamide, galactosamine (*in vitro*)-induced hepatotoxicity[66]. Al-Said *et al.* demonstrated strong anti-inflammatory activity

of *Capparis spinosa*, which was comparable to oxyphenbutazone[67,68]. Bonina *et al*. documented 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*[69]. In another study, Germano *et al*. observed the antioxidant activity of *Capparis spinosa* using tests like lipid peroxidation, bleaching of free radicals and autoxidation of iron ions[70]. Mahasneh *et al.* observed potent antimicrobial and antifungal activity of *Capparis spinosa*[71,72].

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*[73] and Du *et al*. identified the other chemical constituents as alpha-amyrin, taraxerone, baurenyl acetate and beta-sitosterol[74]. Aktay *et al*. and Zafar *et al*. observed 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) [75,76]. Ahmed *et al*. screened *Cichorium intybus* for antihepatotoxic activity and measured the degree of protection using biochemical parameters (AST, ALT, ALKP and TP). Potent antihepatotoxic activity (comparable to Silymarin) was observed with almost complete normalization of the tissues (as neither fatty accumulation nor necrosis was observed on histopathological study)[77]. 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[78]. 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. 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[79]. El *et al*. and Papetti *et al*. documented antioxidative activity (radical scavenging effects, inhibition of hydrogen peroxide, and iron chelation) of *Cichorium intybus*[80,81]. Gurbuz *et al*. observed significant cytoprotection against ethanol-induced damage and these results were further confirmed by using histopathological techniques[82]. Amirghofran *et al*. reported the capacity of *Cichorium intybus* to enhance the proliferation of lymphocytes after stimulation with the allogenic cells[83]. 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[84].

Ikeda *et al.* identified saponins (nigrumnins I and II) as the active ingredients of *Solanum nigrum*[85]. *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)[86]. Sultana *et al*. demonstrated that *Solanum nigrum* protect DNA against oxidative damage and the results suggest that the observed hepatoprotective effect of *Solanum nigrum* might be due to the ability to suppress the oxidative degradation of DNA in the tissue debris[79]. 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 alkaline phosphatase, aspartate aminotransferase and gamma-glutamyltransferase levels in the serum[87]. Son *et al.* reported *Solanum nigrum* as a potent scavenger of hydroxyl radicals and DPPH radicals[88]. 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[89]. Similarly, Akhtar *et al*. observed gastric mucosal cytoprotection offered by *Solanum nigrum* against aspirin-induced gastric ulcers[90]. Qureshi *et al*. reported antifungal activity of *Solanum nigrum*[91].

Upadhyay *et al.* identified arjunetoside, oleanolic and arjunic acids as active ingredients from *Terminalia arjuna*[92]. Munasinghe *et al*. reported potent antioxidant activity of *Terminalia arjuna*, which might be due to its effects on lipid peroxidation[93]. Ali *et al*. demonstrated that arjunaphthanoloside from *Terminalia arjuna* inhibits nitric oxide (NO) production[94] and terminoside A isolated from *Terminalia arjuna,* decreases inducible nitric oxide synthase (iNOS) levels in LPS-stimulated peritoneal macrophages[95]. Cheng *et al*. observed potent antiviral activity by virtue of inhibition of viral attachment and penetration by *Terminalia arjuna*[96]. Samy *et al*. demonstrated potent antibacterial activity of *Terminalia arjuna*[97].

Jafri *et al*. reported significant hepatoprotective effects of *Cassia occidentalis* in chemically induced liver damage[98]. Bin-Hafeez *et al*. showed that *Cassia occidentalis* modulates hepatic enzymes and provides hepatoprotection against induced immunosuppression[99]. Samy *et al*. reported antimicrobial properties of *Cassia occidentalis* comparable with standard reference antibiotics[100]. Perez *et al*. reported strong antibacterial activity of *Cassia occidentalis* against *Salmonella typhi*[101]. Tona *et al*. reported inhibitory effect of *Cassia occidentalis* on *P. falciparum* growth[102]. Caceres *et al*. and Graham *et al*. observed antifungal activity of *Cassia occidentalis*[103,104].

Harnyk *et al*. documented clinically beneficial effects of *Achillea millefolium* in the treatment of chronic hepatitis[105]. Krivenko *et al*. reported similar clinical improvements in chronic hepatocholecystitis and angiocholitis with *Achillea millefolium*[106]. Lin *et al.* observed anti-hepatoma activity of *Achillea millefolium*[107]. Candan *et al*. and Bezic *et al*. reported antioxidant and antimicrobial activities of *Achillea millefolium*[108,109].

Devarshi *et al*. studied Mandur bhasma for the hepatoprotective property in hepatitis induced by CCl_4 and observed prevention of CCl_4 mediated changes in the enzyme activities, which suggests the hepatoprotective role of Mandur bhasma[110].

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

CONCLUSION

In this study, meta-analysis of 50 clinical studies conducted over 30 years in 4490 patients, was performed to evaluate the efficacy and short- and long-term safety of Liv.52 in HA. The cumulative data analysis revealed clinical and biochemical improvements with significant symptomatic control. In addition, there was highly significant reduction in the mean recovery period. There were no reported or observed significant adverse events in all trials and the overall drug compliance was excellent. Therefore, it may be concluded that Liv.52 tablets and syrup are effective and safe in the management of hepatitis A.

REFERENCES

1. Glass, G.V. Primary, secondary and meta-analysis of research. *Educational Researcher* 1976; 5: 351-379.
2. Krugman, S., Ward, R., Giles, J.P. The natural history of infectious hepatitis. *Am J Med* 1962; 32: 717-728.
3. Sule, C.R, Pai, V.R., Damania, R.F., Joshi, V.S. (Miss). Studies with Liv.52 therapy in infective hepatitis. *J Ind Med Prof* 1968; 14: 6391-6397.
4. Jaffari, S.M.H., Shyam Raj. Liv.52 in infective hepatitis. *The Antiseptic* 1969; 5: 353.
5. Arora, A.K. Role of various types of treatment in infectious hepatitis. *Armed Forces Med. J* 1969; 25(3): 362.
6. Doddagoudar. Infective hepatitis - Survey and study of 110 cases. *Probe* 1970; IX(3): 122–123.
7. Lala Surajnandan Prasad, Kaleshwer Prasad. Some observations on Liv.52 in the treatment of infective hepatitis and cirrhosis of liver. *Probe* 1971; X(3): 114-116.
8. Mukerjee, A.B., Dasgupta, M. Chronic active hepatitis (a preliminary observation of 10 cases). *J Ind Med Prof* 1971; 18: 8097.
9. Deshpande, R.S., Shantilal C. Sheth, Joykutty. Infections hepatitis - Study of 100 cases. *Curr Med Pract* 1971; 6: 810.
10. Dasgupta, M., Mukerjee, A.B. Chronic active hepatitis (a preliminary observation of 10 cases). *J Ind Med Prof* 1971; 18: 8097.
11. Patel, G.T., Mruthyunjayanna, B.P., Seetharam, T.D., Channe Gowda, A.C. Liv.52 therapy in viral hepatitis. *Probe* 1972; XI(2): 112-119.
12. Gupta, S., Khatri, R.L., Srivastava, G. Therapeutic effects of Liv.52 in post-necrotic hepatitis. *Probe* 1972; XII(1): 15-20.
13. Ramalingam, V., Sundarvalli, N., Balagopal Raju, V. Liv.52 studies in acute hepatitis. *Indian Pediatrics* 1971; 12: 839.
14. Dave, D.S., Rajput, V.J., Gupta, M.R. Clinico-biochemical study of infective hepatitis with special reference to Liv.52 therapy. *Probe* 1972; XI(4): 214-220.
15. Gupta, S., Khatri, R.L., Srivastava, G. Therapy of infectious hepatitis and other liver disorders. *Probe* 1972; XI(2): 93-99.
16. Sarkar, M.K. Liv.52 in the treatment of acute infective hepatitis. *The Ind Pract* 1973; August: 395.
17. Shishupal Ram, Kumar, P. Observations on the therapy of acute infectious hepatitis with Liv.52. *Probe* 1974; XIV(1): 66-67.
18. Krishnamurthy Leela, Sama, S.K. and Ramachandra, K. Liv.52 in acute virus: A hepatitis - a double-blind trial. XV Annual Conference, Indian Society of Gastroenterology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 8-10 Novermber, 1974, p.2.
19. Reddi, Y.R., Rohini, K., Kusuma, G., Sudhakar Rao, V. Role of Liv.52 and steroids in the management of viral hepatitis in children. *Probe* 1974; XIV(1): 35.
20. Jain, K.C., Khanijo, S.K., Bisarya, B.N. Studies with Liv.52 in infective hepatitis. *Probe* 1974; XIV(1): 64-65.
21. Reddi, Y.R., Sudhakar Rao, V., Rohini, K. The management of viral hepatitis in children. *Indian Pediatrics* 1975; XII(8): 659.
22. Khetarpal, S.K., Veera Kumar, E. Liv.52 therapy in infective hepatitis. *Probe* 1974; XIV(1): 59-63.
23. Sharma, N.L., Lahori, U.C., Mehta, S.K. Studies on Liv.52 in hepatitis disorders (Part I). *Probe* 1974; XIV(1): 54-58.
24. Deshpande, R.S. Acute infectious hepatitis with fulminant hepatic failure – a study of 289 cases. *Probe* 1974; XIV(1): 1-13.
25. Sinha, B.B., Studies on 222 cases of acute infective hepatitis during an epidemic. *Probe* 1974; XIII(3): 130-133.

26. Bhandari, N.R., Shashi Gupta (Miss.). Liv.52 in infective hepatitis. *Probe* 1974; XIV(1): 68-72.
27. Mazumdar, H., Mazumdar, M. Trial of Liv.52 in infections hepatitis in children in Goa. *Probe* 1974; 1: 82.
28. Mitra, D.K., Ashrafuddin, S., Talib, S.H. Liv.52 in viral hepatitis with special reference to its use in precoma and coma. *Probe* 1974; XIV(1): 14-22.
29. Rath, B.B., Sengupta, S.N., Bose, S.N. Preliminary observation on the role of Liv.52 in infective hepatitis with persistent jaundice. *Capsule* 1975; XV(8): 170-175.
30. Agarwal, V.K., Meena Sareen. Clinical trial of Liv.52 drops in infective hepatitis – a comparative study with broad spectrum antibiotics and corticosteroids. *Probe* 1976; XV(2): 112-120.
31. Chawhan, R.N., Talib, S.H., Talib, V.H., Sengupta, S.R., Patil, S.D. Viral hepatitis in children. *The Med & Sur* 1976; 3: 9.
32. Sama, S.K., Krishnamurthy, L., Ramachnadran, K., Krishnan Lal. Efficacy of an indigenous compound preparation Liv.52 in acute viral hepatitis – A double-blind study. *Ind J Med Res* 1976; 5: 738.
33. Singh, D.S., Sama, S.K.,Singh, G. A study of chronic hepatitis in northern India. *Ind J Med Res* 1976; 64(12):1728-1734.
34. Banerjee, L.K., Sahu, N.N.,Ray, S.S., Daljit Singh. Liv.52 therapy in viral hepatitis (A clinico-biochemical study). *Capsule* 1977; 8: 170.
35. Singh, K.K., Singh, Y.K., Sinha, S.K., Sharma, V., Mishra, B.N. Observations on the treatment of infective hepatitis with an indigenous drug Liv.52. *Ind Med Jl* 1977; 5: 69.
36. Habibullah, C.M. Vindod Chandra, Padmanaban, C.G., Ramakrishna, R. Liv.52 in acute viral hepatitis – Results of a double blind study. *The Antiseptic* 1978; 75(8): 491.
37. Mehrotra, M.P., Tandan, S. Liv.52, a clinicobiochemical trial in hepatic cirrhosis. *Curr Med Pract* 1973; 17(4): 185-188.
38. Sethi, J.P., Madhulika Sharma. Clinical management of severe acute hepatic failure with special reference to Liv.52 in therapy. *Probe* 1978; XVII(2): 155-158.
39. Ila V. Desai, Dudhia, M.V., Gandhi, V.K. A clinical study of infective hepatitis treated with Liv.52. *Indian Pediatrics* 1977; 3: 197.
40. Biswas, P. Role of Liv.52 in the treatment of infective hepatitis. *Capsule* 1980; 4: 74.
41. Mandal, U.S. Viral hepatitis and Liv.52. *Capsule* 1980; 3: 50.
42. Shakuntala Saxena (Mrs.), Ashok Kumar Garg, Ashok Jain. Study of Liv.52 therapy in infective hepatitis in children. *Curr Med Pract* 1980; 24(5): 194.
43. Kesarkar, A.S. Liv.52 therapy in acute infective hepatitis. *Capsule* 1981; 4: 74.
44. Chowdhury, A.B. Role of Liv.52 in the treatment of infective hepatitis. *Capsule* 1981; 3: 50.
45. Misra, D.C. Infective hepatitis and Liv.52 drops. *Capsule* 1981; 6: 122.
46. Mandal, J.N., Roy, B.K. Studies with Liv.52 in the treatment of infective hepatitis, chronic active hepatitis and cirrhosis of the liver. *Probe* 1983; XXII(4): 217-242.
47. Sreenivasa Rao, Y. Management of infective hepatitis. *Probe* 1983; XXII(2): 107-109.
48. Bharadia, G.R., Kukade, A.L., Kulkarni, A.S., Rathod, I.M. Liv.52 in the management of various hepatic disorders – A study of 53 cases. *Probe* 1986; XXV(3): 277-280.
49. Vijaykumar, S. Karchi. A trial with Liv.52 in infective hepatitis. *Capsule* 1988; Feb./Mar.: 126.
50. Patney, N.L., Sumanbala Pachori. A study of serum glycolytic enzymes and serum B hepatitis in relation to Liv.52 therapy. *The Med & Sur* 1986; XXVI(4): 9.
51. Dange, S.V., Pawar, S.S., Phadke, S.A., Shrotri, D.S. Comparative efficacy of five indigenous compound formulations in patients of acute viral hepatitis. *Maharashtra Med J* 1989; XXXVI(5): 75.

52. Kalab, M., Krechler, T. Effect of the hepatoprotective drug Liv.52 on liver damage. *The J Czech Physicians* 1997; 136(24): 758-760.
53. Naylor, C.D. Meta-analysis and the meta-epidemiology of clinical research. *BMJ* 1997; 315: 617-619.
54. Bailar, J.C. The promise and problems of meta-analysis [editorial]. *N Engl J Med* 1997; 337: 559-561.
55. Huque, M.F. Experiences with meta-analysis in NDA submissions. *Proceedings of the Biopharmaceutical Section of the American Statistical Association* 1988; 2: 28-33.
56. Chalmers, I., Altman, D.G. Foreword. In: Chalmers I, Altman DG (Eds.) *Systematic reviews*, London: BMJ Publishing, 1995.
57. Dickersin, K., Berlin, J.A. Meta-analysis: state-of-the-science. *Epidemiol Rev* 1992; 14: 154-176.
58. Freiman, J.A., Chalmers, T.C., Smith, H., Kuebler, R.R. The importance of beta, the type II error, and sample size in the design and interpretation of the randomized controlled trial. In: Bailar JC, Mosteller F, (Eds.) *Medical uses of statistics*. Boston, MA: NEJM Books, 1992: p. 357.
59. Lau, J., Antman, E.M., Jimenez-Silva, J., Kupelnick, B., Mosteller, F., Chalmers, T.C. Cumulative meta-analysis of therapeutic trials for myocardial infarction. *N Engl J Med* 1992; 327: 248-254.
60. Jenicek, M. Meta-analysis in medicine. Where we are and where we want to go. *J Clin Epidemiol* 1989; 42: 35-44.
61. Gelber, R.D., Goldhirsch, A. Interpretation of results from subset analyses within overviews of randomized clinical trials. *Stat Med* 1987; 6: 371-378.
62. Collins, R., Keech, A., Peto, R., Sleight, P., Kjekshus, J., Wilhelmsen, L., *et al*. Cholesterol and total mortality: need for larger trials. *BMJ* 1992; 304: 1689.
63. Chalmers, I. Randomised controlled trials of fetal monitoring 1973-1977. In: Thalhammer O, Baumgarten K, Pollak A, (Eds.) *Perinatal medicine*. Stuttgart: Thieme, 1979: p. 260.
64. Smith, Glass, Miller. Does psychotherapy benefit neurotic patients? *Archives of General Psychiatry* 1981; 36: 1203-1208.
65. Khanfar, M.A., Sabri, S.S., Zarga, M.H., Zeller. K.P. The chemical constituents of *Capparis spinosa* of Jordanian origin. *Nat Prod Res* 2003; 17(1): 9-14.
66. Gadgoli, C., Mishra, S.H. Antihepatotoxic activity of p-methoxy benzoic acid from *Capparis spinosa*. *J Ethnopharmacol* 1999; 66(2): 187-192.
67. Al-Said, M.S., Abdelsattar, E.A., Khalifa, S.I., el-Feraly, F.S. Isolation and identification of an anti-inflammatory principle from *Capparis spinosa Pharmazie* 1988; 43(9): 640-641.
68. Ageel, A.M., Parmar, N.S., Mossa, J.S., Al-Yahya, M.A., Al-Said, M.S., Tariq, M. Anti-inflammatory activity of some Saudi Arabian medicinal plants. *Agents Actions* 1986; 17(3-4): 383-384.
69. Bonina, F., Puglia, C., Ventura, D., Aquino, R., Tortora, S., Sacchi, A., Saija, A., Tomaino, A., Pellegrino, M.L., de Caprariis, P. *In vitro* antioxidant and in vivo photoprotective effects of a lyophilized extract of *Capparis spinosa* L buds. *J Cosmet Sci* 2002; 53(6): 321-335.
70. Germano, M.P., De Pasquale, R., D'Angelo, V., Catania, S., Silvari, V., Costa, C. Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source. *J Agric Food Chem* 2002; 50(5): 1168-1171.
71. Mahasneh, A.M. Screening of some indigenous Qatari medicinal plants for antimicrobial activity. *Phytother Res* 2002; 16(8): 751-753.
72. Ali-Shtayeh, M.S., Abu Ghdeib, S.I. Antifungal activity of plant extracts against dermatophytes. *Mycoses* 1999; 42(11-12): 665-672.

73. He, Y., Guo, Y.J., Gao, Y.Y. Studies on chemical constituents of root of *Cichorium intybus*. *Zhongguo Zhong Yao Za Zhi*. 2002; 27(3): 209-210.
74. Du, H., Yuan, S., Jiang, P. Chemical constituents of *Cichorium intybus* L. *Zhongguo Zhong Yao Za Zhi*. 1998; 23(11): 682-683, 704.
75. Aktay, G., Deliorman, D., Ergun, E., Ergun, F., Yesilada, E., Cevik, C. Hepatoprotective effects of Turkish folk remedies on experimental liver injury. *J Ethnopharmacol* 2000; 73(1-2): 121-129.
76. Zafar, R., Mujahid Ali, S. Anti-hepatotoxic effects of root and root callus extracts of Cichorium intybus L. *J Ethnopharmacol* 1998; 63(3): 227-231.
77. Ahmed, B., Al-Howiriny, T.A., Siddiqui, A.B. Antihepatotoxic activity of seeds of *Cichorium intybus*. *J Ethnopharmacol* 2003; 87(2-3): 237-240.
78. Mun, J.H., Woo, Y.J., Jeon, W.H., An, N.H., Park, J.S. Effects of the ethanol extract of *Cichorium intybus* on the immunotoxicity by ethanol in mice. *Int Immunopharmacol* 2002; 2(6): 733-744.
79. Sultana, S., Perwaiz, S., Iqbal, M., Athar, M. Crude extracts of hepatoprotective plants, *Solanum nigrum* and *Cichorium intybus* inhibit free radical-mediated DNA damage. *J Ethnopharmacol* 1995; 45(3): 189-192.
80. El, S.N., Karakaya, S. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. *Int J Food Sci Nutr* 2004; 55(1): 67-74.
81. Papetti, A., Daglia, M., Gazzani, G. Anti- and pro-oxidant activity of water soluble compounds in *Cichorium intybus* var. silvestre (Treviso red chicory). *J Pharm Biomed Anal* 2002; 30(4): 939-945.
82. Gurbuz, I., Ustun, O., Yesilada, E., Sezik, E., Akyurek, N. *In vivo* gastroprotective effects of five Turkish folk remedies against ethanol-induced lesions. *J Ethnopharmacol* 2002; 83(3): 241-244.
83. Amirghofran, Z., Azadbakht, M., Karimi, M.H. Evaluation of the immunomodulatory effects of five herbal plants. *J Ethnopharmacol* 2000; 72(1-2): 167-172.
84. Kim, H.M., Kim, H.W., Lyu, Y.S., Won, J.H., Kim, D.K., Lee, Y.M., Morii, E., Jippo, T., Kitamura, Y., An, N.H. Inhibitory effect of mast cell-mediated immediate-type allergic reactions by *Cichorium intybus*. *Pharmacol Res* 1999; 40(1): 61-65.
85. Ikeda, T., Tsumagari, H., Nohara, T. Steroidal oligoglycosides from *Solanum nigrum*. *Chem Pharm Bull* (Tokyo). 2000; 48(7): 1062-1064.
86. Raju, K., Anbuganapathi, G., Gokulakrishnan, V., Rajkapoor, B., Jayakar, B., Manian, S. Effect of dried fruits of Solanum nigrum LINN against CCl_4-induced hepatic damage in rats. *Biol Pharm Bull* 2003; 26(11): 1618-1619.
87. Moundipa, P.F., Domngang, F.M. Effect of the leafy vegetable *Solanum nigrum* on the activities of some liver drug-metabolising enzymes after aflatoxin B1 treatment in female rats. *Br J Nutr* 1991; 65(1): 81-91.
88. Son, Y.O., Kim, J., Lim, J.C., Chung, Y., Chung, G.H., Lee, J.C. Ripe fruit of *Solanum nigrum* L. inhibits cell growth and induces apoptosis in MCF-7 cells. *Food Chem Toxicol* 2003; 41(10): 1421-1428.
89. Prashanth Kumar, V., Shashidhara, S., Kumar, M.M., Sridhara BY. Cytoprotective role of *Solanum nigrum* against gentamicin-induced kidney cell (Vero cells) damage in vitro. *Fitoterapia* 2001; 72(5): 481-486.
90. Akhtar, M.S., Munir, M. Evaluation of the gastric antiulcerogenic effects of Solanum nigrum, *Brassica oleracea* and *Ocimum basilicum* in rats. *J Ethnopharmacol* 1989; 27(1-2): 163-176.
91. Qureshi, S., Rai, M.K., Agrawal, S.C. *In vitro* evaluation of inhibitory nature of extracts of 18-plant species of Chhindwara against 3-keratinophilic fungi. *Hindustan Antibiot Bull* 1997; 39(1-4): 56-60.

92. Upadhyay, R.K., Pandey, M.B., Jha, R.N., Singh, V.P., Pandey, V.B. Triterpene glycoside from *Terminalia arjuna*. *J Asian Nat Prod Res* 2001; 3(3): 207-212.
93. Munasinghe, T.C., Seneviratne, C.K., Thabrew, M.I., Abeysekera, A.M. Antiradical and antilipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection. *Phytother Res* 2001; 15(6): 519-523.
94. Ali, A., Kaur, G., Hayat, K., Ali, M., Ather, M. A novel naphthanol glycoside from *Terminalia arjuna* with antioxidant and nitric oxide inhibitory activities. *Pharmazie*. 2003; 58(12): 932-934.
95. Ali, A., Kaur, G., Hamid, H., Abdullah, T., Ali, M., Niwa, M., Alam, M.S., Terminoside A a new triterpene glycoside from the bark of *Terminalia arjuna* inhibits nitric oxide production in murine macrophages. *J Asian Nat Prod Res* 2003; 5(2): 137-142.
96. Cheng, H.Y., Lin, C.C., Lin, T.C. Antiherpes simplex virus type 2 activity of casuarinin from the bark of *Terminalia arjuna* Linn. *Antiviral Res* 2002; 55(3): 447-455.
97. Perumal Samy, R., Ignacimuthu, S., Sen, A. Screening of 34 Indian medicinal plants for antibacterial properties. *J Ethnopharmacol* 1998; 62(2): 173-182.
98. Jafri, M.A., Jalis Subhani, M., Javed, K., Singh, S. Hepatoprotective activity of leaves of *Cassia occidentalis* against paracetamol and ethyl alcohol intoxication in rats. *J Ethnopharmacol* 1999; 66(3): 355-361.
99. Bin-Hafeez, B., Ahmad, I., Haque, R., Raisuddin, S. Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice. *J Ethnopharmacol* 2001; 75(1): 13-18.
100. Samy, R.P., Ignacimuthu, S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. *J Ethnopharmacol* 2000; 69(1): 63-71.
101. Perez, C., Anesini, C. *In vitro* antibacterial activity of Argentine folk medicinal plants against *Salmonella typhi*. *J Ethnopharmacol* 1994; 44(1): 41-46.
102. Tona, L., Ngimbi, N.P., Tsakala, M., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T., Pieters, L., Totte, J., Vlietinck, A.J. Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. *J Ethnopharmacol* 1999; 68(1-3): 193-203.
103. Caceres, A., Lopez, B., Juarez, X., del Aguila, J., Garcia, S. Plants used in Guatemala for the treatment of dermatophytic infections. 2. Evaluation of antifungal activity of seven American plants. *J Ethnopharmacol* 1993; 40(3): 207-213.
104. Graham, J.G., Zhang, H., Pendland, S.L., Santarsiero, B.D., Mesecar, A.D., Cabieses, F., Farnsworth, N.R. Antimycobacterial Naphthopyrones from *Senna obliqua*. *J Nat Prod* 2004; 67(2): 225-227.
105. Harnyk, T.P. The use of preparations of plant origin in treating and rehabilitating elderly patients with chronic hepatitis. *Lik Sprava*. 1999; 7-8: 168-170.
106. Krivenko, V.V., Potebnia, G.P., Loiko, V.V. Experience in treating digestive organ diseases with medicinal plants. *Vrach. Delo*. 1989; 3: 76-78.
107. Lin, L.T., Liu, L.T., Chiang, L.C., Lin, C.C. *In vitro* anti-hepatoma activity of fifteen natural medicines from Canada. *Phytother Res* 2002; 16(5): 440-444.
108. Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sokmen, A., Akpulat, H.A. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. millefolium Afan. (Asteraceae). *J Ethnopharmacol* 2003; 87(2-3): 215-220.
109. Bezic, N., Skocibusic, M., Dunkic, V., Radonic, A. Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. *Phytother Res* 2003; 17(9): 1037-1040.

110. Devarshi, P., Kanase, A., Kanase, R., Mane, S., Patil, S., Varute, A.T. Effect of Mandura bhasma on lipolytic activities of liver, kidney and adipose tissue of albino rat during CCl_4 induced hepatic injury. *J Biosciences* 1986; 10(2): 227-234.