COMPARATIVE STUDY OF THE HEPATOPROTECTIVE EFFICACY OF A FEW MARKETED POLYHERBAL PRODUCTS

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Summary

Research project deals with the comparison of three major polyherbal formulations, which are available in market in India for hepatoprotective efficacy. The formulations used were Livfit, Livomyn and Liv-52. These formulations were compared for their hepatoprotective efficacy with the standardized extract of *Phyllanthus niruri,* a known hepatoprotective agent, which is also one of the components of two out of the three formulations used in the study. The acute animal models selected were carbon tetrachloride induced liver injury, paracetamol induced liver toxicity and thioacetamide induced liver necrosis in rats. A chronic model was also carried out using carbon tetrachloride as hepatotoxic agent. The liver damage was assessed by the estimation of biochemical serum enzyme markers, liver weight and histopathological studies. In carbon tetrachloride induced acute liver damage, only *Phyllanthus niruri* extract and Livfit were effective in reducing ALT Levels. In paracetamol induced liver damage, all treatments were effective in preventing liver damage. In thioacetamide model, *Phyllanthus niruri* and livomyn showed best action while in chronic model; all treatments were effective in reducing the liver damage. From the results, it was concluded that *Phyllanthus niruri* was effective in most of the models tested and out of the three formulations used Livfit showed better activity compared to other two formulations.

Keywords: *Phyllanthus niruri*, Livfit, Livomyn, Liv-52, Hepatoprotection.

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Introduction

Liver diseases have become a global problem. The principal causative factor for the liver diseases in developed countries is chronic alcoholism, while in the developing countries the most frequent causes are malnutrition, anemia, infection and availability of hepatotoxic drugs over the counter. Hepatoxicity manifest as necrosis and cirrhosis. Hardly any effective measures are available for the treatment of liver diseases viz, corticosteroids, anti-viral and immunosuppressant agents are sometimes inadequate and may lead to serious adverse effects[1]. In India numerous medicinal plants are used to treat hepatic diseases. Several formulations made from these plants are available in the market. Some of the constituents present in these formulations are same while others are different. Many of the plants present in these formulations are reported to have hepatoprotective activity while a few of them are included in the formulations although no documented report is available to indicate their hepatoprotective activity. It is not known whether the amount of the active ingredients of each plant present in the formulations will show any hepatoprotective activity. Moreover, the hepatoprotective activities of these formulations are reported either by the company itself or by the institutes that have been sponsored by the company to carry out the work on these formulations. Since the hepatoprotective activities of these formulations are not carried out by any independent organization and the formulations available in the market are not standardized for their chemical composition, we planned to carry out the comparative efficacy of these formulations in experimentally induced liver damage in rats. The three best selling hepatoprotective products in the market were selected for the present study.

Methods

Extracts: *Phyllanthus niruri* extract was obtained from Phytotech Extracts situated in Bangalore.

Selection of dose and treatment period for acute models: The treatment period consisted of 10 days in all the acute models. The following doses were administered once daily for duration mentioned above.
Phyllanthus niruri group- 142.5 mg/Kg body weight of rat[2] .
Livfit, Livomyn and Liv-52 group- 2.85 ml/Kg body weight of rat (calculated based on human dose from the label).

Experimental animals: Male albino Wistar rats weighing between 200-250 gm were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

***Evaluation of hepatoprotective activity (Acute hepatitis model)*:**
Carbon tetrachloride induced acute hepatic injury:
Drugs and Chemicals: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) & Bilirubin estimations kits (Agappe company kits), carbon tetrachloride (Ranbaxy, Delhi) and liquid paraffin (CDH, Mumbai). All chemicals used were of analytical grade.

Treatment protocol:
Group 1: Normal group: Animal of this group received normal saline, p.o. 2.85 ml/Kg daily for ten days.
Group 2: Control group: Animals of this group received normal saline, 2.85 ml/Kg daily p.o. for ten days.
Group 3: *Phyllanthus niruri* group: Animals of this group received *Phyllanthus niruri*, 142.5 mg/Kg/day, p.o. for ten days.
Group 4: Livfit group: Animals of this group received Livfit 2.85 ml/kg daily, p.o. for ten days.
Group 5: Livomyn group: Animals of this group received Livomyn 2.85 ml/kg daily, p.o. for ten days.
Group 6: Liv-52 group: Animals of this group received Liv-52, 2.85 ml/kg daily, p.o. for ten days.

Food was withdrawn 12hrs before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2, 3, 4, 5 and 6. Single dose of CCl_4 (0.5 ml/kg, p.o.) diluted with liquid paraffin (1:1) was administered on 10^{th} day and sacrificed 24 hrs after the administration of CCl_4. Blood samples were collected by retro orbital puncture method and serum was used for AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and serum bilirubin estimation. The liver was isolated and was washed with normal saline, blotted with filter paper and weighed immediately[3]. Liver was sliced and pieces were preserved in 10% formaldehyde solution for histopathological study.

Paracetamol induced liver toxicity:
Treatment protocol: Treatment groups are same as CCl_4 induced acute hepatic injury.
Food was withdrawn 12 hrs before paracetamol administration to enhance the acute liver toxicity in animals of group 2, 3, 4, 5 and 6. Paracetamol (2 g/Kg, p.o.) diluted with sucrose solution (40%w/v) was administered in 3 divided doses and animals were sacrificed 48 hrs after administration of Paracetamol[4]. Blood samples were collected by retro orbital puncture method and serum was used for AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and serum bilirubin estimation. The liver was isolated and was washed with normal saline, blotted with filter paper and weighed immediately. Liver was sliced and pieces were preserved in 10% formaldehyde solution for histopathological study.

Thioacetamide induced liver necrosis: Treatment groups are same as CCl_4 induced acute hepatic injury. Single dose of Thioacetamide (100 mg/kg, s.c.) diluted with distilled water (5% solution) was administered on ninth day to groups 2, 3, 4, 5 and 6 and sacrificed 48hrs after administration of thioacetamide[4]. Blood samples were collected by retro orbital puncture method and serum was used for AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and serum bilirubin estimation. The liver was isolated and was washed with normal saline, blotted with filter paper and weighed immediately. Liver was sliced and pieces were preserved in 10% formaldehyde solution for histopathological study.

***Evaluation of hepatoprotective activity (Chronic hepatitis model)*:**
Carbon tetrachloride induced acute hepatic injury:
Treatment protocol:

Group 1: Normal group: Animal of this group received normal saline, p.o. 2.85 ml/Kg daily for eight weeks.
Group 2: Control group: Animals of this group received normal saline, 2.85 ml/Kg daily p.o. for eight weeks.
Group 3: *Phyllanthus niruri* group: Animals of this group received *Phyllanthus niruri*, 142.5 mg/Kg/day, p.o. for eight weeks.
Group 4: Livfit group: Animals of this group received Livfit 2.85 ml/kg daily, p.o. for eight weeks.
Group 5: Livomyn group: Animals of this group received Livomyn 2.85 ml/kg daily, p.o. for eight weeks.
Group 6: Liv-52 group: Animals of this group received Liv-52, 2.85 ml/kg daily, p.o. for eight weeks.

Dose of CCl_4 (0.2 ml/kg, p.o) diluted with liquid paraffin (1:1) was administered twice weekly to groups 2, 3, 4, 5 and 6. Eight weeks after CCl_4 administration started, and 24 hours after the last treatment, the rats were sacrificed[5]. Blood samples were collected by the retro orbital puncture method and serum was used for AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and serum bilirubin estimation. The liver was harvested, washed in normal saline, blotted with filter paper and weighed immediately. Liver was sliced and pieces were preserved in 10% formaldehyde solution for histopathological study.

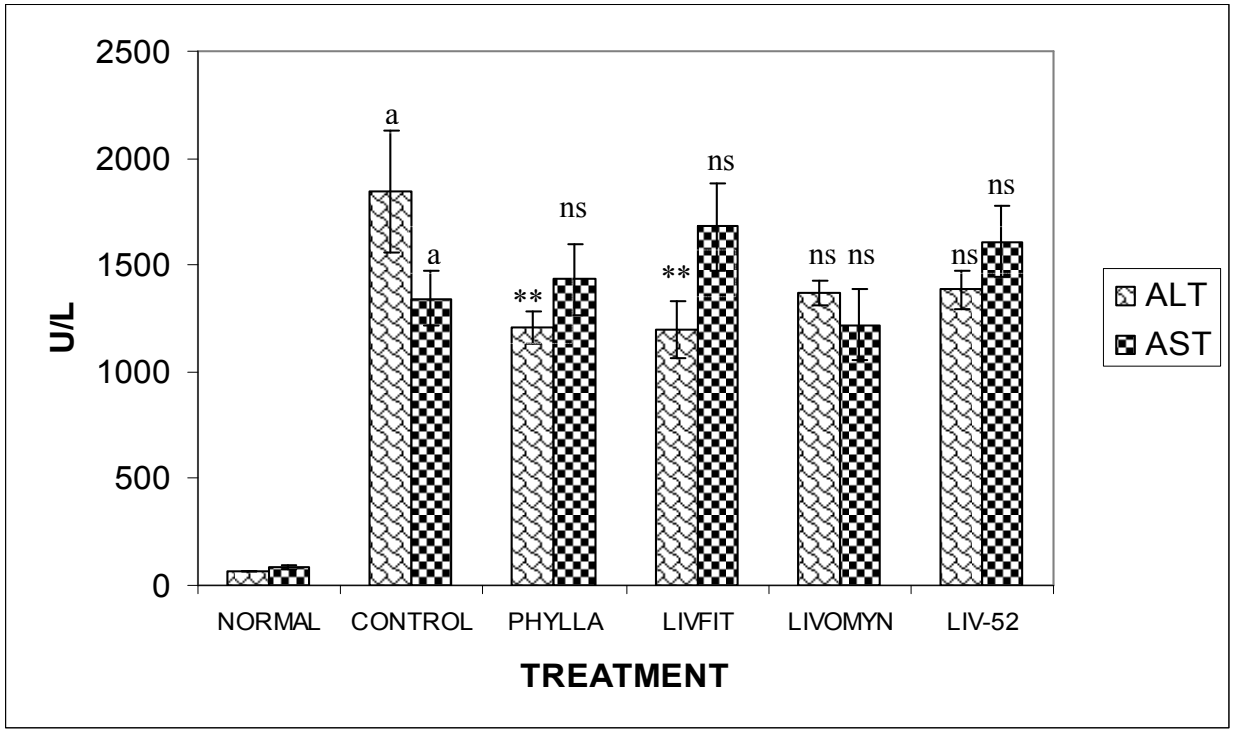
Statistical analysis
The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. The values are expressed as mean $\pm$ SEM and $p<0.05$ was considered significant.

Results

Carbon tetrachloride induced acute hepatic injury: A significant difference in biochemical markers was observed between normal and CCl_4 control groups. Comparative analysis on the effect of ALT and AST revealed that *Phyllanthus niruri* extract and livfit reduced the ALT levels significantly. But Livomyn and Liv-52 did not show significant reduction. *Phyllanthus niruri* increased the bilrubin levels compared to control. The other drugs did not show significant difference in the bilirubin levels. (fig: 1, 2). Administration of CCl_4 has produced a non significant increase in liver weight. None of the treated groups affected the liver weight significantly when compared to control. (fig: 3). Histopathology of normal animals, liver sections showed lobules of hepatocytes oriented around the central vein.

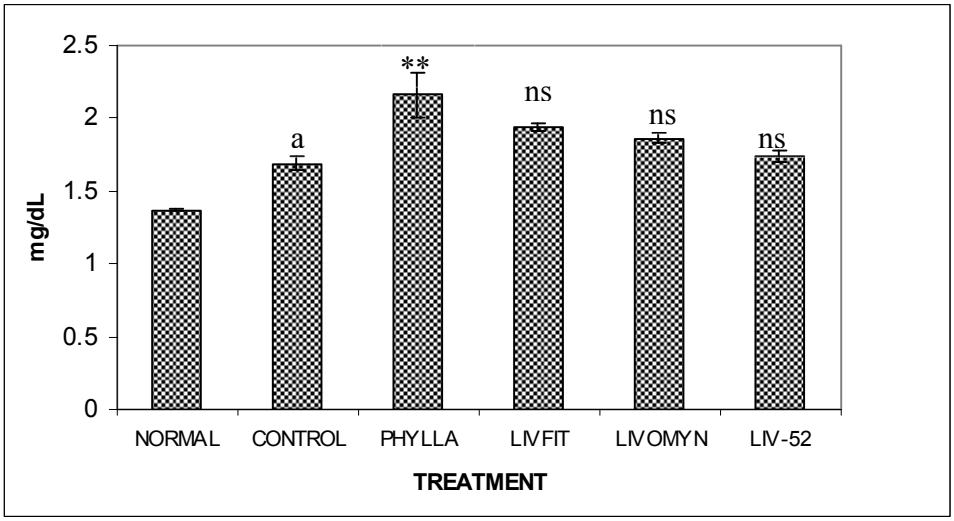
The hepatocytes are arranged in "plates" or anastomosing sheets. The hepatocytes are polygonal cells with well preserved cytoplasm, uninucleated with prominent nuclei. Between the cords of hepatocytes are vascular sinusoids.In CCl_4 treated control animals liver sections showed lobular disarray, loss of architecture, ballooning, centrilobular necrosis, bridging necrosis, periportal microvascular fatty change, sinusoidal dilatation, hyperplasia, crowding of central vein and mild portal inflammation. In *Phyllanthus niruri* treated animals liver sections showed the same effect as in the control group except lesser crowding of central vein, no bridging necrosis and mild necrosis compared to control group. In livfit treated animals liver sections showed almost the same effect as in the control group. In livomyn treated animals liver sections showed the same effect as in the control group except lesser crowding of central vein, no bridging necrosis and mild necrosis compared to the control group. In Liv-52 treated animals liver sections showed the same effect as in the control group except moderate necrosis.

Fig 1: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on ALT and AST in CCl_4 induced acute hepatitis in rats.



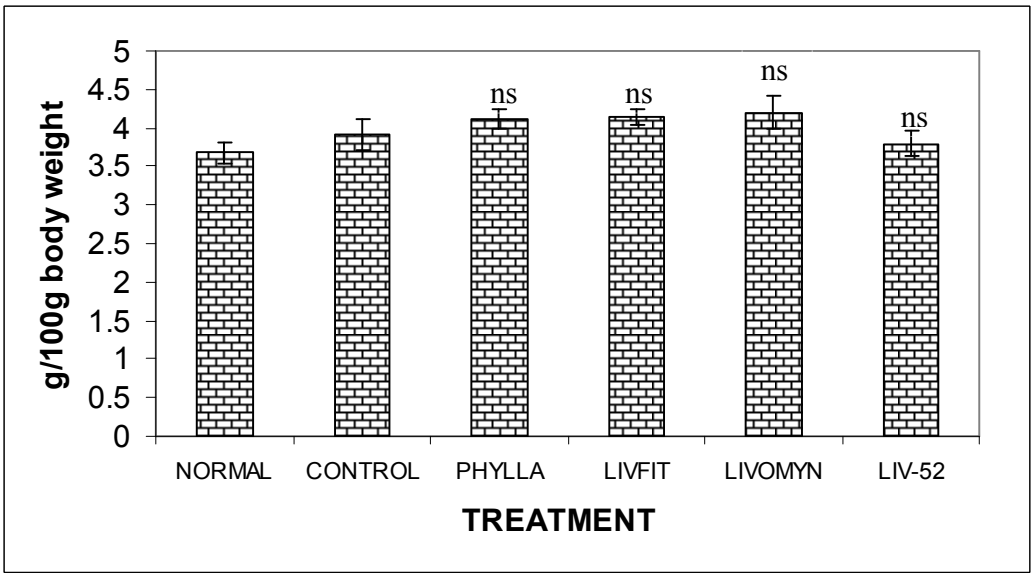
Values are mean ± S.E.M, n = 6, [a] P>0.01 vs. normal animals, [**] P<0.01, [ns] P>0.05 vs control animals.

Fig 2: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on serum bilirubin levels in CCl_4 induced acute hepatitis in rats.



Values are mean ± S.E.M, n = 6, [a] P>0.01 vs. normal animals, [**] P<0.01, [ns] P>0.05 vs control animals.

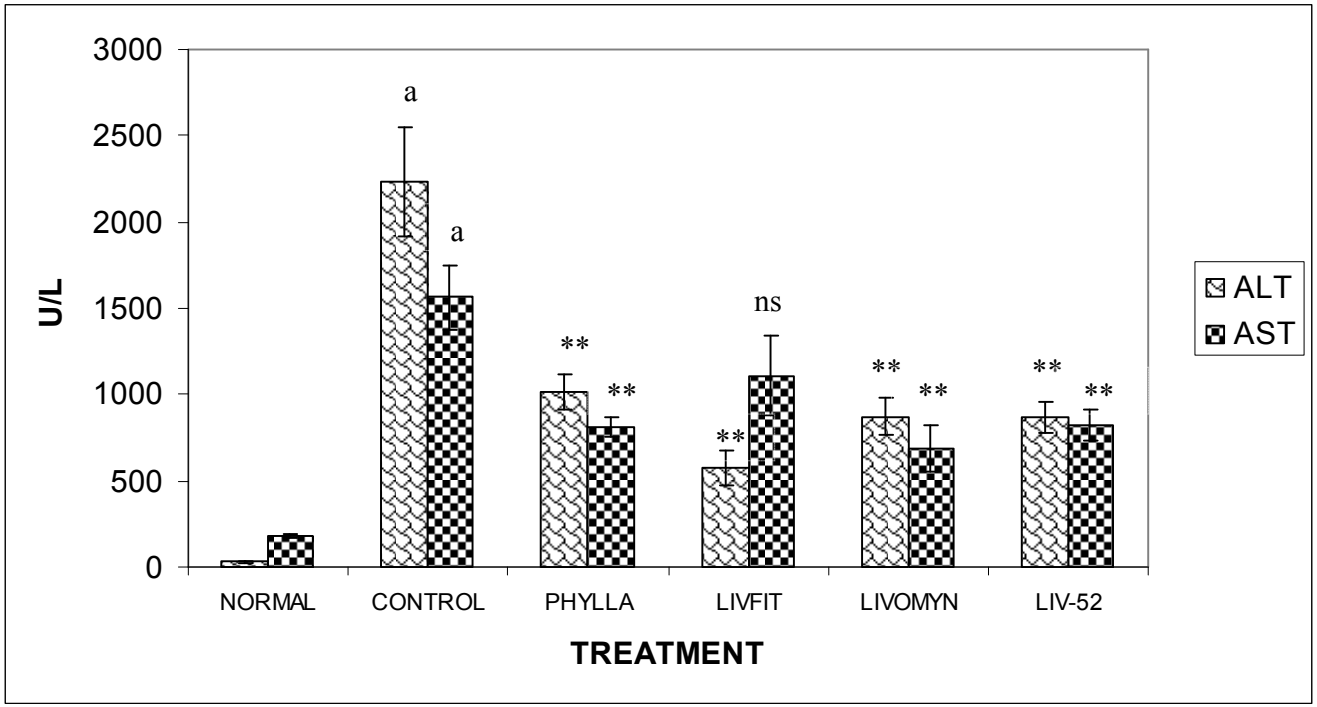
Fig 3: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on liver weight in CCl_4 induced acute hepatitis in rats.



Values are mean ± S.E.M, n = 6, [ns] P>0.05 vs control animals.

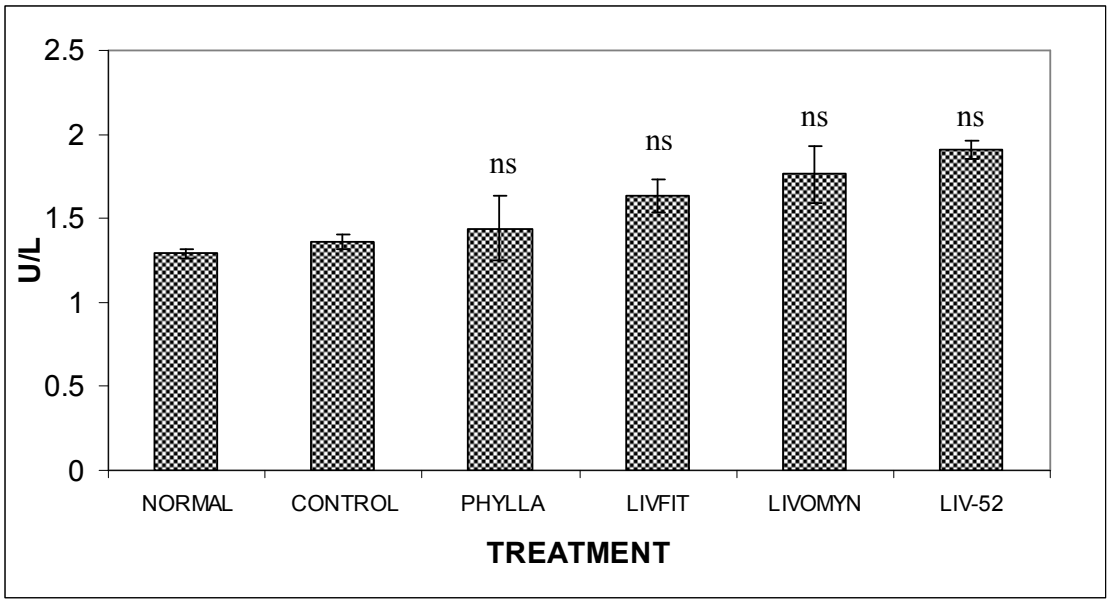
Paracetamol induced liver toxicity: Forty eight hours after treatment with paracetamol, the parameters ALT and AST in the serum increased remarkably. *Phyllanthus niruri*, Livomyn, and Liv-52 reduced ALT and AST levels significantly compared to paracetamol treated group and the decrease in the levels were similar. In animals pretreated with Livfit, the ALT levels decreased significantly. Serum bilirubin levels were not affected by drug treatment significantly (fig: 4, 5). Paracetamol acute toxicity had increased the liver weight. Pretreatment with *Phyllanthus niruri* and Livfit decreased the liver weight significantly (fig: 6). The decrease in liver weight in livomyn and Liv-52 pretreated groups were non significant. In histopathology of normal animals, liver sections showed lobules of hepatocytes oriented around the central vein. The hepatocytes are arranged in "plates" or anastomosing sheets. The hepatocytes are polygonal cells with well preserved cytoplasm, uninucleated with prominent nuclei. Between the cords of hepatocytes are vascular sinusoids. In paracetamol treated control animals liver sections showed lobular disarray, loss of architecture, ballooning, extensive centrilobular necrosis, bridging necrosis, periportal microvascular fatty change, sinusoidal dilatation, more hyperplasia, crowding of central vein and mild portal inflammation. In *Phyllanthus niruri* treated animals liver sections showed almost similar to normal liver. However it showed loss of architecture, lobular disarray and mild necrosis. In Livfit and Livomyn treated animals liver sections showed similar to normal liver. In Liv-52 animals liver sections showed similar to control except it had normal architecture, absence of lobular disarray, mild necrosis, no crowding of central vein, lesser sinusoidal dialatation and lesser hyperplasia .

Fig 4: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on ALT and AST level in paracetamol (PCM) induced acute hepatotoxicity in rats.



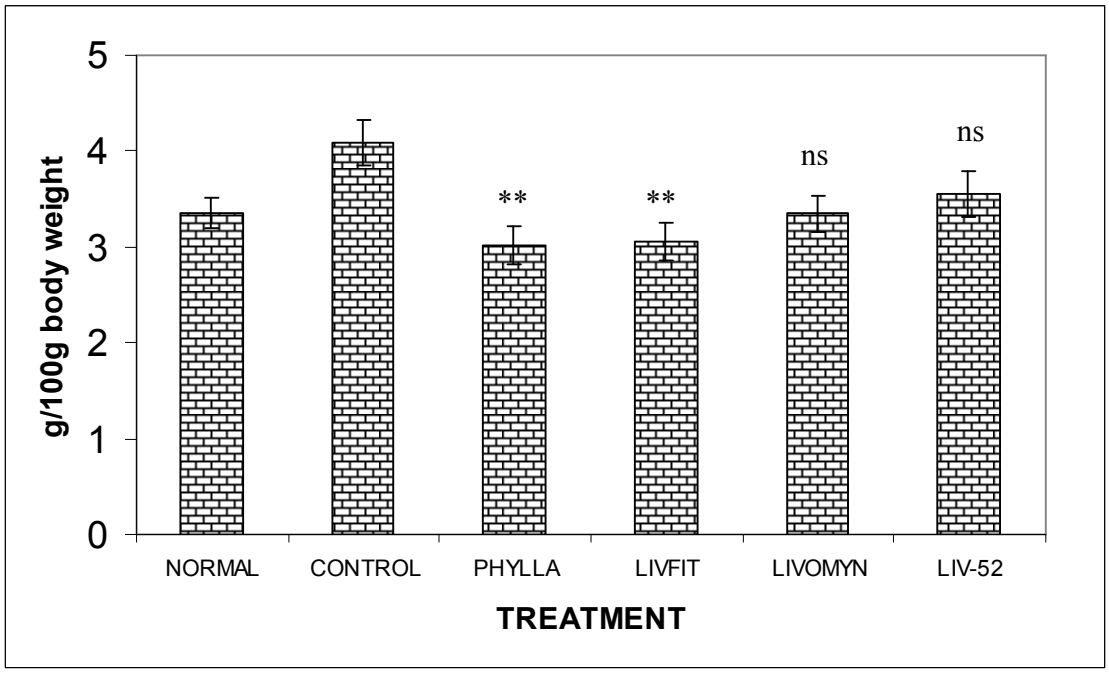
Values are mean ± S.E.M, n = 6, [a] P>0.01 vs. normal animals, [**] P<0.01, [ns] P>0.05 vs control animals.

Fig 5: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on serum bilirubin level in paracetamol induced acute hepatotoxicity in rats.



Values are mean ± S.E.M, n = 6, ^{ns} P>0.05 vs control animals.

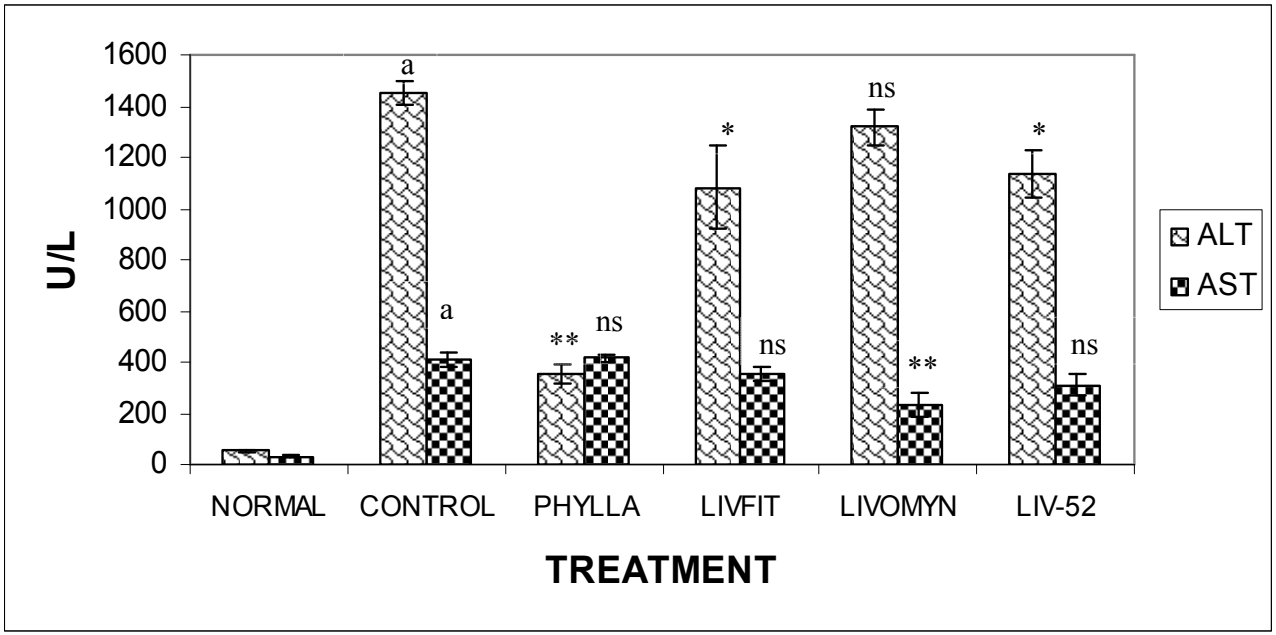
Fig 6: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on liver weight in paracetamol induced acute hepatotoxicity in rats.



Values are mean ± S.E.M, n = 6, ** P<0.01, ^{ns} P>0.05 vs control animals

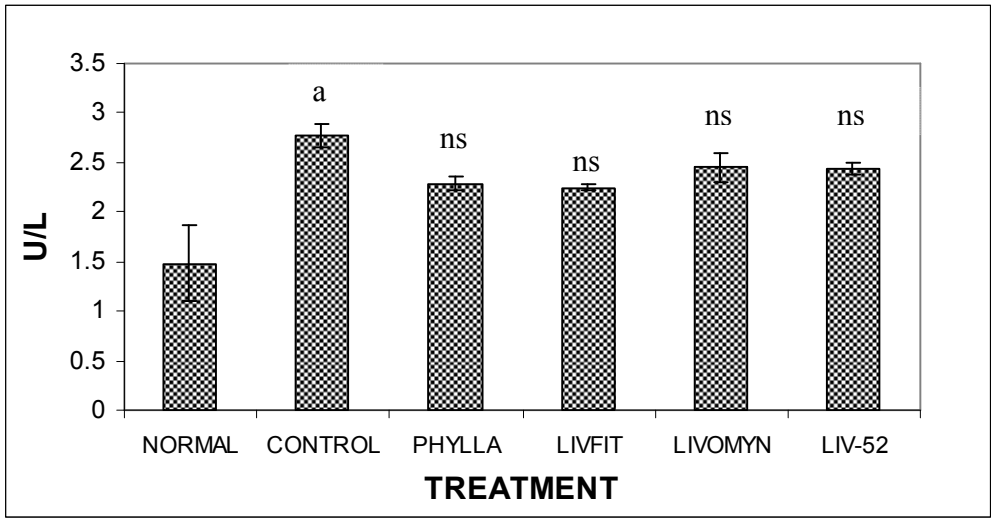
Thioacetamide induced liver necrosis: *Phyllanthus niruri* reduced the ALT levels and Livomyn reduced the AST levels significantly. Livfit and Liv-52 shown lesser effect on ALT level compared to *Phyllanthus niruri* and Livomyn. None of the treatments showed any significant reduction in serum bilirubin levels (fig: 7, 8). *Phyllanthus niruri* decrease the liver weight significantly when compared to control. Livfit, Livomyn and Liv-52 treated animals liver weight did not show any significant decrease (fig: 9). Histopathological analysis of thioacetamide treated control animals liver sections showed lobular disarray, loss of architecture, ballooning, centrilobular necrosis, bridging necrosis, periportal microvascular fatty change, sinusoidal dilatation, hyperplasia, crowding of central vein, bile duct proliferation, bridging fibrosis, regeneration and mild portal inflammation. In *Phyllanthus niruri* treated animals liver sections showed the same effect as in the control group except microvascular fatty change, less sinusoidal dilatation, less hyperplasia and less regeneration. ln Livfit treated animals liver sections showed similar effect as in control group except lesser sinusoidal dilatation, lesser hyperplasia and lesser regeneration. In Livomyn treated animals liver sections showed similar effect as in control group except diffused microvascular fat, lesser hyperplasia, mild crowding of central vein, no bile duct proliferation, no regeneration and mild fibrosis. In Liv-52 treated groups liver sections showed similar effect as in control group except portal inflammation, increased fibrosis, lesser sinusoidal dilatation, microvascular fat and increased regeneration.

Fig 7: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on Serum ALT and AST in thioacetamide (TAA) induced acute hepatic necrosis in rats.



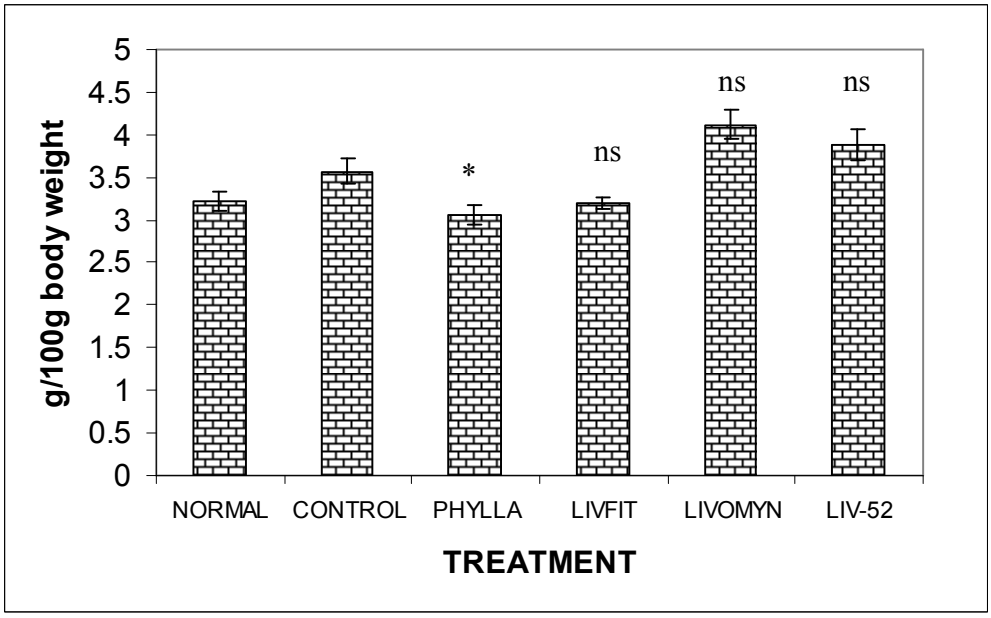
Values are mean ± S.E.M, n = 6, [a] P<0.001 vs. normal animals, * P<0.05, ** P<0.01, [ns] P>0.05 vs. control animals

Fig 8: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on serum bilirubin level in thioacetamide induced acute necrosis in rats.



Values are mean ± S.E.M, n = 6, [a]p>0.05 vs. normal animals, [ns]p>0.05 vs. control animals.

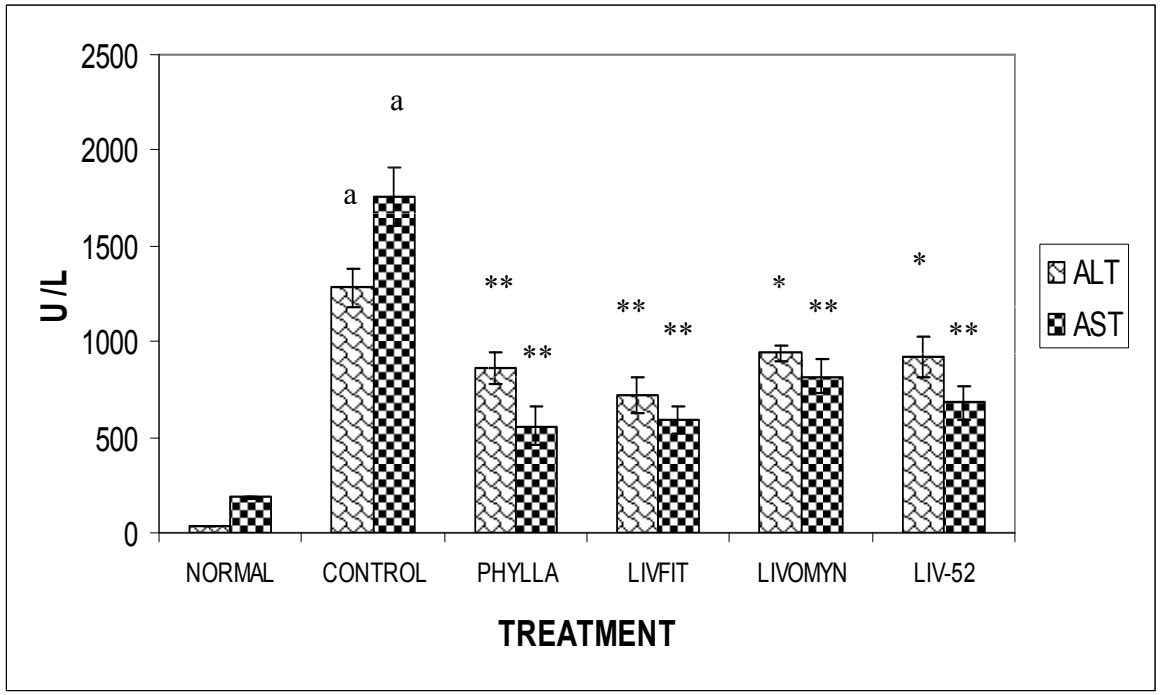
Fig 9: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on liver weight in thioacetamide induced acute necrosis in rats.



Values are mean ± S.E.M, n = 6,* P<0.05, [ns] P>0.05 vs control animals.

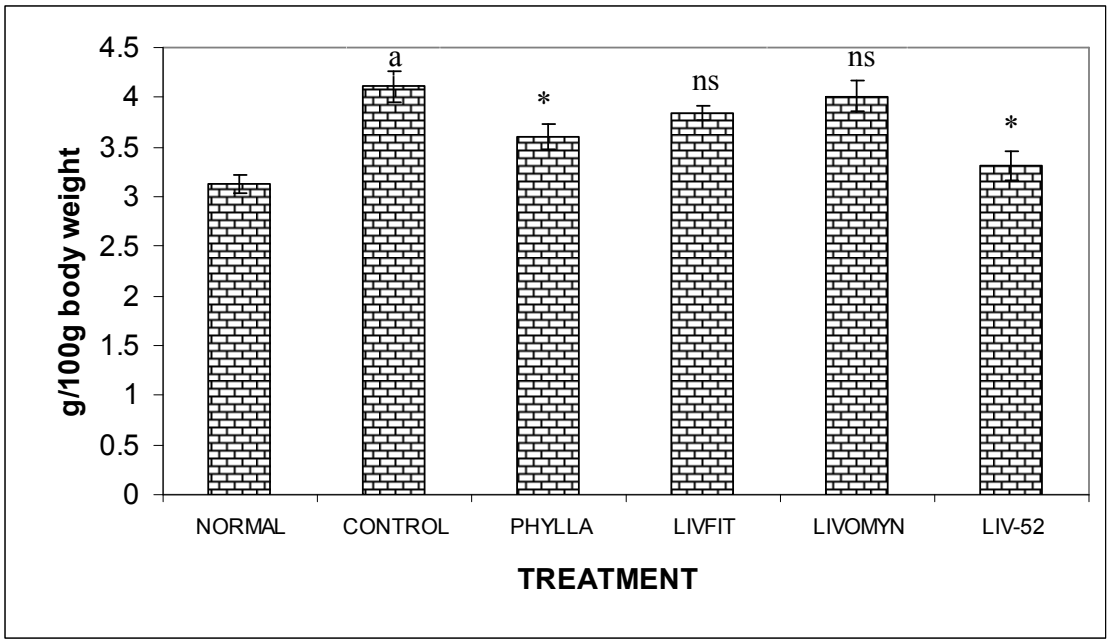
Carbon tetrachloride induced chronic hepatic injury: A significant difference in biochemical markers was observed between normal and CCl_4 treated groups. Unlike effect on acute models in the chronic model, all the formulations and *Phyllanthus niruri* extract showed significant reduction in ALT and AST levels when compared to control. (fig: 10). Administration of CCl_4 increased the liver weight of control significantly compared with normal group. *Phyllanthus niruri* and Liv-52 reduced the liver weights significantly compared to control.(fig: 11). Normal histology of liver showed sinusoidal architecture of hepatocytes having no sign of necrosis or degeneration. In CCl_4 treated control animals the liver section showed loss of architecture, lobular disarray, diffuse piecemeal necrosis, fatty change, sinusoidal dilatation, hyperplasia, crowding of central vein, portal inflammation, bile duct proliferation, fibrosis, regeneration and cirrhosis. In *Phyllanthus niruri* and livfit treated animals the liver sections showed similar effects as in control except more crowding of central vein and more hyperplasia. In livomyn and liv-52 treated animals the liver sections showed similar effects as in control.

Fig 10: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on Serum ALT and AST in CCl_4 induced chronic hepatitis in rats



Values are mean ± S.E.M, n = 6, [a] $p<0.01$ vs. normal animals. [*] $p<0.05$, [**] $p<0.01$ vs. control animals.

Fig 11: Effect of *Phyllanthus niruri*, Livfit, Livomyn, Liv-52 on liver weight in CCl_4 induced chronic hepatitis in rats.



Values are mean ± S.E.M, n = 6, $^{a}p<0.01$ vs. normal animals, $^{*}p<0.05$, $^{ns}p>0.05$ vs control animals.

Discussion

The study was taken up with an aim to determine the efficacy of marketed hepatoprotective agents. The present work dealt with the comparative study of three best selling polyherbal hepatoprotective formulations; Livfit, Livomyn and Liv-52. A monoherbal standardized extract of *Phyllanthus niruri* was also evaluated for hepatoprotective activity. *Phyllanthus niruri* is one of the constituent of two formulations; Livfit and Livomyn. The monoherbal extract of *Phyllanthus niruri* was used to determine whether addition of many plants in a formulation is superior to the use of one single herb for hepatoprotection.

From the results, it is evident *Phyllanthus niruri* produced better effect compared to the three polyherbal products. Among the polyherbal formulations, Livfit was slightly more effective than other two products.

In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity[6,7,8,9]. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations[7,8,9]. Some of the polyherbal formulations are verified for their hepatoprotective action against chemical induced liver damage in experimental animals[7,8,9,10,11]. In most of these studies, marginal or moderated levels of hepatoprotective activities were observed. It is believed that efficacy is not sufficient enough to use these agents as effective drugs[12].

Besides, most of the reported studies described the beneficial effects of the drugs against few hepatotoxic chemical-induced subclinical level of hepatotoxicity. It is not known whether or not these drugs exhibit any beneficial effects against severe liver damage.

Further, the herbal products manufactured in India have been criticized by regulatory agencies in both India and abroad. A recent study conducted by 'National Commission of Macroeconomics and Health (NCMH)' set up by the Ministry of Health to review the state of the country's health, concluded that "Liv-52 is a useless liver drug" [13]. Another report published by Dhiman and Chawla[14] also reported that Liv 52 is not effective for treatment of alcohol induced liver damage. However, *Phyllanthus niruri* was reported to be effective. There are no studies conducted on Livfit and Livomyn by independent organization that are not supported by the manufacturers.

The present study was carried out using four different models of liver damage to determine the effects of drugs. The different drugs used in the present study showed variable effects in different models of hepatotoxicity. This difference could probably be due to the different mechanisms by which hepatotoxicity is induced.

In acute CCl_4 model, injury produced by CCl_4 is mediated by a selective metabolite like trichloromethyl free radicle (CCl_3^-) formed by the haemolytic cleavage of CCl_4 or by an even more reactive species like trichloro peroxy free radicle (Cl_3COO^-) formed by the reaction of CCl_3 and O_2 [15]. In case of CCl_4 (0.5 ml/kg b. wt) intoxicated rats in acute model, the extract of *Phyllanthus niruri* (142.5 mg/kg b. wt) and Livfit (2.85 ml/kg b. wt) prevented the increase of ALT levels and but did not prevent the increase in other biochemical marker levels and liver weight.

Paracetamol is well-known antipyretic and analgesic agent, which produces hepatotoxicity in higher doses. Paracetamol damages liver by covalent binding of its toxic metabolite N-acetyl-p-benzoquinoneimine to sulphydryl group of proteins resulting in lipid peroxidation induced by a decrease in glutathione in the liver[16,17]. This is evidenced by an elevation in the liver weight and serum ALT, AST and bilirubin levels. In experimental animals pretreated with *Phyllanthus niruri*, Livomyn and Liv-52 (2.85 ml/kg b. wt) the ALT and AST levels were significantly lowered. Animals pretreated with Liv-52 (2.85 ml/Kg body weight) had significantly reduced the serum bilirubin levels. *Phyllanthus niruri* and Livfit had shown to prevent increase in liver weight significantly in liver of rats pretreated with paracetamol whereas the other formulations could not prevent the increase in liver weight. Histopathological observation in experimental animals pretreated with Livfit and Livomyn, showed similar to that of normal liver.

Thioacetamide interferes with the movement of RNA from the nucleus to cytoplasm which may cause membrane injury resulting in rise in serum biochemical markers[18]. *Phyllanthus niruri* and Livomyn produced a decrease in AST levels significantly. Livfit and Liv-52 pretreated animals shown to decrease the ALT levels significantly. *Phyllanthus niruri* and Livomyn pretreated animals shown to decrease the liver weight significantly. In histopathology Livomyn and Livfit shown lesser degenerative changes compared to other drugs. In short, *Phyllanthus niruri* and Livomyn showed better activity than the other formulations in acute thioacetamide model.

In chronic CCl_4 model, *Phyllanthus niruri* and Livfit decreased the ALT and AST levels significantly. Livomyn and Liv-52 shown less significance in decreasing ALT levels but they shown same effect in decreasing AST levels. The liver weight of *Phyllanthus niruri* and Liv-52 pretreated animals decreased significantly. In histopathology all the groups showed the same changes that of the control. In short *Phyllanthus niruri* and Livfit had shown better activity than other formulations in chronic CCl_4 model.

The present study substantiates the earlier reports by NCMH and Dhiman and Chawla[14] that Liv-52 may not be very effective. In the present study, Liv-52 showed effect only in paracetamol induced liver damage and chronic damage induced by CCl_4 and comparing the overall results, it can be suggested that Liv-52 was least effective among the three polyherbal products. The monoherbal extract of *Phyllanthus niruri* was the most effective and this substantiates the early study conducted by Dhiman & Chawla[14] on *Phyllanthus niruri.*

Hence, from the results of the study, it is recommended that the manufacturers should concentrate on using standardized monoherbal extracts containing known amount of active constituent than mixing more than one drug in the formulation. Further, it is recommended that standardization of herbal drugs, proper preclinical studies and clinical studies should be carried out before marketing the herbal drugs.

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