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**(Research Article)**

# Comparative Study of Hepatoprotective Activity of Proprietary Polyherbal Preparations against Paracetamol Induced Toxicity

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**ABSTRACT**

*Despite the widespread use of polyherbal formulations, there is a lack of scientific evidence on their efficacy and safety. The present study was designed to evaluate the comparative hepatoprotective activity of three proprietary polyherbal formulations Liv-52, Livomyn and Livosin in acute liver toxicity in rat model induced by Paracetamol. Four groups of 6 albino wistar rats each were subjected to experimental study. Group 1 was given single dose of Paracetamol 500mg/kg orally on day 22, Group 2,3 and 4 were administered with 1.5 ml Liv-52, Livomyn and Livosin twice daily respectively for 21 days, then they were administered with Paracetamol single dose 500mg/kg orally on day 22. The hepatoprotective effect of these polyherbal preparations were evaluated by the assay of liver function biochemical parameters like Serum Glutamic Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP) and Total Bilirubin (TB) levels. The results obtained have shown that the polyherbal hepatoprotective formulations Liv-52 and Livomyn were most effective at the dose of 0.15 ml/Rat. It was found that there was a significant decrease in the serum levels of SGPT, ALP and Total Bilirubin of Liv-52 and Livomyn whereas Livosin was found to be less effective, which justify their use as a hepatoprotective agent. The present study demonstrated that Liv 52 and Livomyn were more effective when compared to Livosin.*

**Key Words:** SGPT, ALP (Alkaline Phosphatase), Total Bilirubin, Liv 52, Livomyn, Livosin.

## INTRODUCTION

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI)[1], which causes oxidative stress[2]and Glutathione (GSH) depletion[3]. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome $P_{450}$. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for Paracetamol induced hepatotoxicity[4].

Liver plays a vital role in the metabolism and elimination of various exogenous and endogenous compounds. As a result of its continuous involvement, it is susceptible to toxic injuries caused by certain agents and any damage to hepatic cells disturb body metabolism. Liver is responsible for metabolism of chemicals and foods for the regulation of internal environment[5]. The major functions of the liver are detoxification of carbohydrates, proteins and fat metabolism[6], secretion of bile and storage of vitamins etc. Excess consumption of certain toxic chemicals[7] such as antibiotics, chemotherapeutics, peroxidised oils, acetaminophen, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons[8], alcohol etc. produces toxic metabolites which damage the liver. Modern medicines have little to offer for alleviation of hepatic diseases[5]and it is chiefly the plant based preparations which are employed for the treatment of liver disorders[9].

For a long time, medicinal plants and their extracts were widely used in the treatment of liver diseases like hepatitis[10] and liver cirrhosis. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activity[11]. In recent times lot of interest has been generated to find out a natural remedy for hepatic disorders caused by toxins like alcohol[12] and hepatitis virus[13].The agent should protect against such damage, especially of one which facilitates regeneration[14]by proliferation of parenchymal cells after damage[8]and arrest growth of fibrous tissue[11]. There is no remedy for liver diseases[15] which are so prevalent in the population. Nearly 150 phytoconstituents[5,10]

from 101 plants have been claimed to possess liver protecting activity[16]. In the traditional system of medicines, many medicinal plants have been reported to possess the potential to treat liver diseases[17] but the treatment is mainly symptomatic[18].

Livosin, an indigenous polyherbal preparation, has been widely used as a hepatoprotective agent in various liver disorders[15]. Composition of Livosin: Each 5 ml of Livosin contains Triphala (60 mg), Kalmegh (50 mg), Hansapadi (50 mg), Bhrigaraj (50 mg), Pudina (50 mg), Sonapata (50 mg), Carica papaya (50 mg), Anantamool (50 mg), Ashwagandha (50 mg), Arjuna (50 mg), Kurchi (50 mg) etc.

Livomyn, a polyherbal preparation is found to have hepatoprotective activity when used in liver disorders[19]. Each 5ml of Livomyn contains *Phyllanthus niruri* (100mg), Triphala (90mg), *Amoora rohituka*, *Boerhaavia diffusa* and C*ichorium intibus* each 75mg, *Zingiber officinalis* (35mg), *Berberis aristata* (25mg), *Tephrosia Perpurea* (15mg), *Aloe barbadensis* (30mg).

LIV-52, a hepatotonic has shown protective effects in hepatotoxicity induced by radiations and widely employed as a reliable product for hepatoprotection[20]. Each 5 ml of Liv.52 syrup contains *Capparis Spinosa* (34 mg), *Cichorium intybus* (34 mg), *Solanum nigrum* (16mg), *Cassia occidentalis* (8 mg), *Terminalia arjuna* (16 mg), *Achillea millefolium* (8 mg) and *Tamarix gallica*(8 mg)[21]. It has been reported that Liv-52 protects liver from the hepatotoxicity of Paracetamol, anticancer drugs[22], antibiotics, oral contraceptives, alcohol, allyl alcohol, and carbon tetrachloride[23]. We have done this study to find out which one is the most effective hepatoprotective among the said marketed preparation. This study was designed and executed with sole intention of finding out the most effective, reliable hepatoprotective available in market.

## MATERIALS AND METHODS

Albino Wistar rats, 130-150 gm of either sex obtained from the animal house of CMR College of Pharmacy, Hyderabad. The work has been approved from Institution Animal Ethical Committee (1657/PO/a/12/CPCSEA). Animals were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic conditions under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given *ad libitum*. The bedding material of the cages were changed every day. After one week of acclimatization, animals were randomly divided into five groups of six rats (n=6) each. Paracetamol (PCM) was given orally single dose 500mg/Kg body weight on day 22 to groups 2-5 to induce hepatotoxicity. LIV-52 (Himalaya Pharmaceuticals), Livomyn(Charak Pharmaceuticals), Livosin(Ayur Pharmaceuticals) were used. Animals in Group 1, 2, and 3 were administered 1.5 ml LIV-52, Livomyn and Livosin respectively twice daily per oral for 21 days while group 4 received 1.5 ml of normal saline (See Table 1). The hepatoprotective effect of these polyherbal preparations were evaluated by the assay of liver function biochemical parameters[24]like Serum Glutamic Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP) and Total Bilirubin (TB) activities).

### Assessment of Hepatoprotective Activity

*Collection of blood samples*

The blood samples were collected on 22$^{nd}$ day under light ether anesthesia through retro orbital puncture route, collected blood samples were stored in cool conditions. Clear serum was obtained by centrifuging blood samples at rate of 1200g for 10 minutes and assessed for the levels of Serum Glutamic Pyruvate Transeaminase (SGPT), Alkaline Phosphatase (ALP) and Total Bilirubin (TB)[25].

*Estimation of SGPT*

Solution 1 composed of 1 ml 4N NaOH, diluted to 10 ml with distilled water was made and kept aside. Buffered alanine-KG substrate (Reagent 1), DNPH (2,3 Di nitro phenyl hydrazine) color reagent (Reagent 2)and sodium hydroxide (Reagent 3) are ready for use as such[26] (Reitman S et al; 1957). 0.5ml buffered alanine-KG substrate was taken in test tube and incubated at 37$^{o}$C for 5 minutes, serum 0.1 ml was added to it and incubated at 37$^{o}$C for 30 min, followed by addition of 0.5 ml DNPH color reagent which was allowed to stand at room temperature for 20 minutes. 5ml of solution 1 was added to the solution of the test tube, mixed well and absorbance of the solution was measured at 505 nm using water as blank after leaving at room temperature for 10 minutes.

*Estimation of Bilirubin*

3 ml of Total Bilirubin reagent (Reagent A) and 0.1 ml of Direct Bilirubin reagent (Reagent B) were mixed by inversion, after 30 seconds serum 0.15 ml was added to it and incubated for 37$^{o}$C for 5 minutes ensuring the uniformity of contents in mixture. Absorbance was noted at 540 nm using water as blank. For direct Bilirubin estimation, 3 ml of Direct Bilirubin reagent and 0.1 ml of sodium nitrite reagent (Reagent C) were mixed by inversion and waited for 30 seconds. Serum 0.15 ml was added to it and content was mixed well which was incubated for 37$^{o}$C for 5minutes. Absorbance was read at 540 nm using water as blank. The absorbance of the artificial standard (Reagent D = 10mg % Bilirubin) was read directly against distilled water[26].

Calculation Formulae for Serum Bilirubin in mg % :

$$\text{Total Bilirubin (A)} = \frac{(\text{Absorbance of T} - \text{Absorbance of TB}) \times 10}{\text{Absorbance of Standard}}$$

$$\text{Direct Bilirubin (B)} = \frac{(\text{Absorbance of D} - \text{Absorbance of DB}) \times 10}{\text{Absorbance of Standard}}$$

Where T= Total Bilirubin, TB= Total Bilirubin blank, D= Direct Bilirubin, DB= Direct Bilirubin blank.

*Estimation of Alkaline Phosphatase (ALP)*

The substrate, p-nitrophenyl phosphate (PNPP) is hydrolyzed by ALP to p-nitrophenol and phosphoric acid. Some divalent ions like $Mg^{++}$ are added to the system which acts as activators. PNPP is colourless in acid or alkaline medium while PNP is yellow in colour in the alkaline medium and colourless in the acid medium. Glycine buffer was used for maintaining the pH of the reaction medium.

Reagents employed were:

1. Stock substrate of PNPP (4 mg per ml): 0.4 g of p - nitrophenyl disodium phosphate was dissolved in 100 ml water.
2. Sodium Hydroxide Solutions:
1N NaOH was obtained by dissolving 40 g NaOH in 800 ml of water and volume made one liter by diluting it with water. Other strengths (0.1 N, 0.05 N, and 0.02 N) were made by diluting1N NaOH with in the ration of 1:10, 1:20 and 1:50 to obtain 0.1 N, 0.05 N, and 0.02 N NaOH solutions. 10 ml, 5 ml and 2 ml of 1N NaOH was taken separately from that and each was diluted to 100 ml that yielded NaOH solutions of above strengths in the sequence.
3. Glycine buffered substrate:
(i) Glycine buffer (alkaline):7.5 g of glycine, 0.095 g of magnesium chloride, 750 ml water, and 85 ml 1N sodium hydroxide were mixed thoroughly and the solution was diluted to one liter and refrigerated.
(ii) Working substrate: Equal volume of glycine buffer and stock substrate of PNPP were mixed and pH was adjusted to 10.3 -10.4 only when it was needed.
4. Standard solution of p-nitrophenol (PNPP):
(i) Stock Standard (1mmole/L):139.1 mg of high purity PNP was made 1000 ml solution by diluting with water in a 1-litre volumetric flask. This solution was stable only when stored in dark.
(ii) PNP working standard (0.04 mmole/L): This solution was prepared daily for the test by diluting1.0 ml of the stock standard with 0.05 N NaOH solutions.

**Procedure**

1.0 ml of buffered substrate was taken in each of the two test tubes, labeled as "T" and "B" corresponding to test and blank. The blank "B" was the serum blank. Tubes were placed in a water bath set at 37$^{o}$C for 5 to 7 minutes to equilibrate the temperature. With the timer set, 0.05 ml serum was added to the "T" tube and mixed. Tubes were incubated at 37$^{o}$C for exactly 30 minutes. Upon completion of 30 minutes, 10 ml of 0.05 M NaOH was added to both test tubes to stop the reaction and formed PNP was diluted and mixed well. 0.05 ml of serum was added to the B tube (serum blank), and contents were mixed thoroughly. The absorbance for contents of both B and T were read at 405 nm against water as instrument blank[26].

## RESULTS AND DISCUSSION

As evident from biochemical parameters, PCM (500 mg/kg Body weight) given once orally showed hepatotoxicity after 24 hrs. PCM treatment significantly increased the serum liver enzyme levels, viz., SGPT, ALP and total bilirubin. The activity of SGPT (321.00 ± 87.93 IU/l), ALP (257.50 ± 17.64 IU/l),Total bilirubin (2.02±0.03) was significantly higher ($P$<0.05) in PCM treated group in comparison to normal control (ALT 33.33±0.61; ALP 152.17±11.40 IU/l, total bilirubin 0.82±0.06) indicating a marked hepatocellular injury (See Table-1). Three polyherbal formulations namely Liv 52, Livosin, Livomyn were given for 21 days prior to PCM. The pre-treatment with Liv52 at a dose of 0.15ml/rat significantly ($P$<0.05) reduced SGPT,Total bilirubin and ALP values. As compared to Liv 52 (100%), there was a 104 per cent reduction in serum levels of SGPT in Livomyn pretreated group. The significant lowering of enzyme levels was observed particularly in Liv52 and Livomyn group. However, Livosin group showed similar but slightly lesser effects in comparison to the groups of Liv 52 and Livomyn. The values are expressed as mean ± SEM (n= 6 mice/ group). $P$<0.05 compared to normal control group, Liv 52, Livosin, Livomyn treated group by One-way ANOVA followed by Student-Newman-Keuls test. Percentage reduction in different parameters is compared to Liv 52 and is given in brackets considering the difference between PCM and Liv 52 treatment groups as 100 per cent.

PCM treatment significantly increased the serum enzyme levels, namely ALT, Total bilirubin and ALP indicating chemical induced hepatocellular toxicity. Serum levels of these enzymes are very sensitive markers employed in the diagnosis of liver diseases. When the hepatocellular plasma membrane is damaged, these enzymes normally present in the cytosol are released into the blood stream. This can be quantified to assess the type and extent of liver injury. ALP is excreted normally via bile by the liver. Serum Glutamic Pyruvic Transaminase (SGPT) also called as Alanine transaminase (ALT) and it is also referred or Alanine amino transferase (ALAT) is an enzyme present in hepatocytes. Elevations of SGPT are often measured in multiples of the upper limit of normal (ULN) and is required to diagnose acute liver damage, such as viral hepatitis or Paracetamol overdose. The estimation of total and direct Bilirubin is of importance for diagnosis and differentiation of jaundice. The serum levels of unconjugated Bilirubin rises in the cases of hemolytic jaundice. Whereas conjugated serum Bilirubin levels rises in the cases of obstructive jaundice. Hepatic jaundice is characterized by simultaneous rise in both, conjugated and unconjugated serum Bilirubin levels.The liver injury due to toxins can result in defective excretion of bile by hepatocytes which are reflected as their increased levels in serum[8]. Pretreatment with polyherbal formulations restored the liver enzyme parameters[27,17]. The significant reduction of liver enzyme parameters like SGPT, TB and ALP is manifests extent of liver injury due to toxic drugs, alcohol and virus[28.] The protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage caused by PCM.

**Table 1:** Effect of pretreatment with different polyherbal formulations (Dose 0.15ml/Rat BID) on serum levels of Liver Enzymes

| Sr. No. | Groups | SGPT (IU/l) (% Reduction) | ALP (IU/l) (% Reduction) | TB (mg/dl) |
|---|---|---|---|---|
| **1** | PCM | 321.0 ± 87.93 | 257.50 ± 17.64 | 2.02±0.03 |
| **2** | Liv 52+ PCM | 113.17 ± 16.60 (100) | 165.83 ± 11.24 (100) | 0.90009±0.0516 (94.16) |
| **3** | Livomyn+ PCM | 105.67 ± 16.65 (104) | 220.83 ± 22.33 (40) | 1.66±0.295 (85.83) |
| **4** | Livosin+ PCM | 195.33 ± 15.68 (61) | 231.33± 33.57 (29) | 1.75 ±0.608 (49.99) |

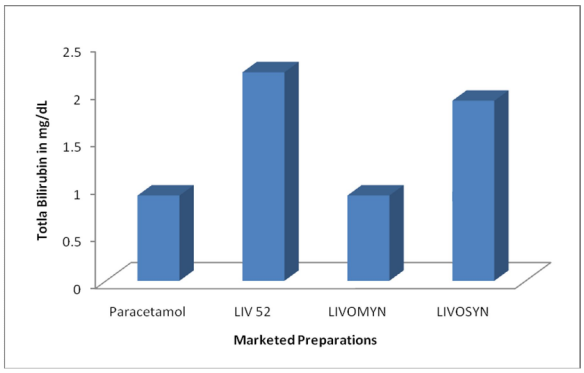


**Figure 1:** Effect of Polyherbal formulations on Total Bilirubin

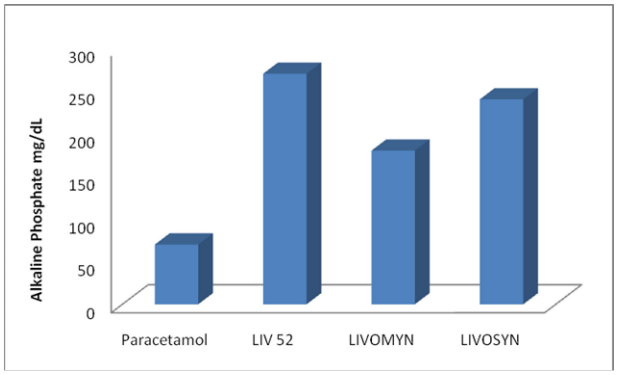


**Figure 2:** Effect of Polyherbal formulations on Alkaline Phosphatase

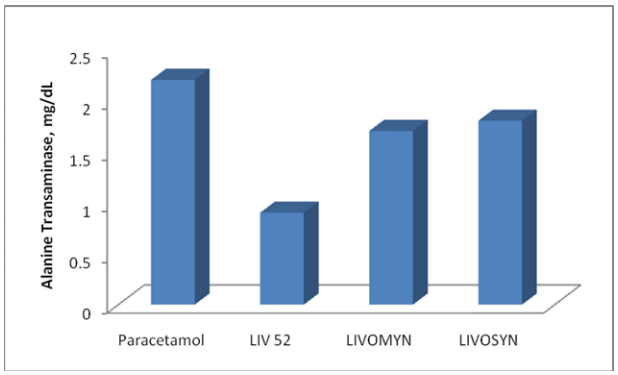


**Figure 3:** Effect of Polyherbal formulations on Alanine Transaminase.

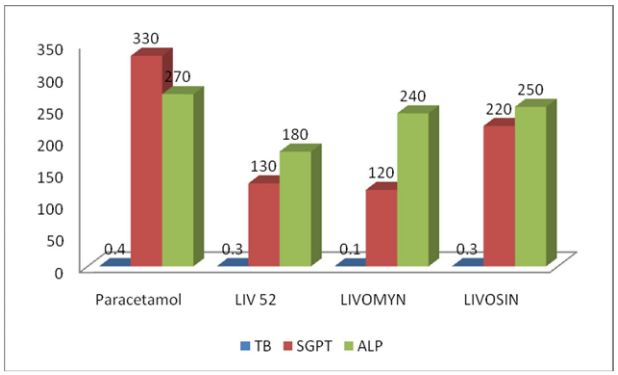


**Figure 4:** Effect of Various Polyherbal formulations on TB, SGPT and ALP

## CONCLUSION

Effect of all the 3 formulations have been assessed for level of ALT, ALP and Total Bilirubin. The results obtained from the study have shown that polyherbal hepatoprotective formulations Liv 52 and Livomyn were most effective at the dose of 0.15 ml/Rat twice a day. In the present study there was a significant decrease in the serum level of ALT, ALP and Total Bilirubin of Liv 52 and Livomyn whereas Livosin was found to be less effective, which justify their use as a hepatoprotective agent. Results obtained above makes us to conclude that Liv 52 and Livomyn are of same therapeutic efficacy while Livosin has lesser therapeutic efficacy as a hepatoprotective.

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