

# PRECLINICAL EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF THE POLYHERBAL FORMULATION ‘LIVOPICK’

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## ABSTRACT

The present study was aimed to evaluate the hepatoprotective effect polyherbal formulation livopick in acute experimental liver injury induced by paracetamol in rats.

In all models, rats of either sex were pre-treated with livopick 150mg/kg and 100mg/kg a low and high dose orally. The protective effect of prophylactic treatment was analysed by estimation of serum biomarkers like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (total and direct) and also by histopathological observation. The dose of paracetamol was given orally. When compared with toxic control, the activities of serum biomarkers in all prophylactic treated groups were significantly decreased. It was concluded that the polyherbal formulation livopick has more potent hepatoprotective effect which is evident by the reduction in the elevated marker enzyme level and which was also supported by antioxidant study and histopathological study.

**Key words:** Livopick, hepatoprotective, antioxidant.

## INTRODUCTION

Liver is the most important organ in the human body. It plays a supreme role in the metabolism of xenobiotics, drugs and in the regulation of homeostasis. Liver plays an important role in detoxification and excretion of many endogenous and exogenous compounds. In addition to its metabolic function it is able to store and release of variety of endogenous substrate, vitamins, minerals, etc.<sup>1</sup>

However liver is one of the most frequently injured organs in the body. A number of hepatotoxins such as viruses, bacteria, chemicals, medicines and alcohol targets the liver and causes liver injury. This leads to the manifestation of various liver disorders such as hepatitis, alcoholic liver disease, non-alcoholic liver disease, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.<sup>2</sup>

It is well recognized that free radicals are critically involved in liver diseases. Hepatotoxicants promote formation of free radicals and consequent lipid peroxidation damages the membranes of liver cells and organelles and causing the swelling and necrosis of hepatocytes<sup>3</sup>. This becomes responsible for the release of cytosolic enzymes into the blood. The magnitude of derangement of liver hepatotoxins is generally measured by the level of serum enzyme biomarkers and antioxidants<sup>4</sup>.

Medicinal plants and their derivatives are still used all over the world in one form or another as a remedy for the various diseases. Alternative and traditional medicines have scores of advantages over the conventional medicines.<sup>5</sup>

Livopick is a polyherbal formulation. The principle ingredients of livopick are powder and extract of *Rheum emodi*, *Picrorhiza kurroa*, *Plumbago zeylanica*, *Phyllanthus niruri*, *Andrographis paniculata*, *Eclipta alba*, *Boerhaavia diffusa*, *Tinospora cordifolia* and *Triphala* (Equal parts of *Terminalia chebula* extract, *Terminalia bellerica* extract, *Embllica officinalis* extract). Individually all the above is proved to be hepatoprotective antioxidants, asthma, hepatoprotective, antidiabetic, antihistaminic, antibacterial antipyretic, anti-ulcer, diuretics, anticancer properties<sup>6-12</sup>. But no scientific report is available for its combined hepatoprotective effect.

Hence the present study has been designed to evaluate hepatoprotective activity of the polyherbal formulation ‘Livopick’ against the pathological condition of hepatic system induced by paracetamol induced experimental models in *Albino* rats.

## Materials and methods

### Experimental Animals:

Albino Wister rats of either sex weighing 150-250g were housed at  $25^{\circ} \pm 5^{\circ}\text{C}$ , relative humidity  $50 \pm 5\%$  in a well-ventilated animal house under 12:12 hr light dark cycle. The animals were maintained under standard conditions in the animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol. (SDCP/IAEC-12/2012-13)

**Drug and Dosage:** Polyherbal formulation LIVOPICK (Wilson Drugs and Pharmaceutical Pvt. Ltd, India), paracetamol (National chemicals, Gujarat). Chemicals and reagents were procured from standard companies. The doses which were administered to rats were calculated accordingly from human dose. The human dose was converted to rat dose by using Human equivalent dose method (HED)<sup>13</sup>. The doses of high dose and low dose of livopick were selected as 150mg/kg/day and 100mg/kg/day.

### Experimental Protocol:

The animals were divided into 5 groups consisting of six animals each. Group I and Group II were used as Normal control and toxic control respectively and treated with normal saline by oral route. The group III, IV and V treated with silymarin 100 mg/kg (standard), livopick 150mg/kg (high dose), livopick 100mg/kg (low dose) respectively. All treatments were given for once daily for 5 days by oral route. Apart from normal control all other treatment groups were treated with paracetamol 2g/kg after dilution with 40% w/v sucrose on day 5 to induce hepatotoxicity. Food was withdrawn 12 hours before paracetamol administration to enhance liver damage in animals of all groups except normal control. The animals were sacrificed 48 hrs after the administration of paracetamol. Blood samples were collected by retro-orbital puncture method and serum was used for assay of marker enzymes. Then the animals were sacrificed and the liver from each group were isolated and washed with normal saline, dried using filtered paper and weighed immediately and subjected for histopathological studies.<sup>14</sup>

**Biochemical analysis:** Using commercially available kits (Robonik India Pvt Ltd, Mumbai), serum levels Aspartate transaminase (AST) and Alanine transaminase (ALT) alkaline phosphatase (ALP) and serum bilirubin (total & direct) were quantified according to the manufacturer's guidelines using Autoanalyser (Robonik, Mumbai). Antioxidant levels such as superoxide dismutase (SOD), Glutathione (GSH) and Catalase were estimated from Liver tissue homogenate.

### Histological studies

Liver from each group were isolated and fixed immediately in 10% neutral formalin solution. The liver sections were stained with hematoxylin and eosin and histological changes were observed microscopically

### Statistical analysis:

Statistical analysis was done using Graph Pad Prism version 4 software (Graph Pad Inc, USA). ANOVA followed by Bonferroni's Multiple Comparison test (compare all) was applied. Data was presented as MEAN $\pm$ SEM. P<0.05 was considered significant.

## RESULTS:

### Effect on serum biomarkers:

- **Effect on Alanine aminotransferase (ALT):**

There was an extremely significant ( $p < 0.001$ ) increase in ALT level in toxic control when compared to that of normal control. The pre-treatment of standard silymarin, both high dose and low dose of Livopick exhibited an extremely significant ( $P < 0.001$ ) decrease in ALT levels compared to toxic control (Table 1).

- **Effect on Aspartate aminotransferase (AST):**

There was an extremely significant ( $p < 0.001$ ) increase in AST level in toxic control when compared to that of normal control. The pre-treatment of standard silymarin, both high dose and low dose of Livopick exhibited an extremely significant ( $P < 0.001$ ) decrease in AST levels compared to toxic control (Table 1).

- **Effect on Alkaline phosphatase (ALP):**

There was an extremely significant ( $p < 0.001$ ) increase in ALP level in toxic control when compared to that of normal control. The pre-treatment of standard silymarin, both high dose and low dose of Livopick exhibited an extremely significant ( $P < 0.001$ ) decrease in ALP levels compared to toxic control (Table 1).

- **Effect on Total bilirubin:**

There was an extremely significant ( $p < 0.001$ ) increase in total bilirubin level in toxic control when compared to that of normal control. The pre-treatment of standard silymarin and high dose of Livopick exhibited an extremely significant ( $P < 0.001$ ) decrease in total bilirubin levels compared to toxic control. Low dose treated

group revealed moderately significant ( $P < 0.01$ ) decreased total bilirubin level compared to toxic control (Table 2).

- **Effect on Direct bilirubin:**

There was an extremely significant ( $p < 0.001$ ) increase in direct bilirubin level in toxic control when compared to that of normal control. The pre-treatment of standard silymarin and high dose of Livopick exhibited an extremely significant ( $P < 0.001$ ) decrease in direct bilirubin levels compared to toxic control. The low dose treated group revealed moderately significant ( $P < 0.01$ ) decreased levels of direct bilirubin compared to toxic control (Table 2).

**Effect on Antioxidants:**

- **Effect on GSH:**

In this experiment model, the toxic control revealed an extremely significant ( $p < 0.001$ ) decrease in GSH levels when compared to the normal control. Prior treatment with silymarin and high dose of livopick showed extremely significant ( $p < 0.001$ ) and low dose treated group demonstrated moderately significant ( $p < 0.01$ ) increase in GSH levels when compared with toxic control (Table 3).

- **Effect on Catalase :**

In this experiment model, the toxic control revealed an extremely significant ( $p < 0.001$ ) decrease in catalase activity when compared to the normal control. Prior treatment with silymarin, high dose and low dose of livopick showed extremely significant ( $p < 0.001$ ) elevation in catalase activity when compared with Toxic control (Table 3).

- **Effect on SOD:**

In this experiment model, the toxic control revealed an extremely significant ( $p < 0.001$ ) decrease in SOD levels when compared to the normal control. Prior treatment with silymarin, high dose and low dose of livopick showed moderately significant ( $p < 0.01$ ) increase in SOD activity when compared with Toxic control (Table 3).

Table 1: Effect of Livopick on serum ALT, AST, ALP in PCM induced liver toxicity in rats.

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control	52.24±1.62	127.34±1.83	295.56±1.78
Toxic control (PCM)	302.85±2.14 <sup>***</sup>	458.71±1.26 <sup>***</sup>	678.78±2.56 <sup>***</sup>
Silymarin (100mg/kg) (STD)	59.92±1.34 <sup>###</sup>	151.19±2.67 <sup>###</sup>	363.87±3.57 <sup>###</sup>
Livopick (150mg/kg) (HD)	95.23±2.12 <sup>###</sup>	165.26±6.53 <sup>###</sup>	398.34±3.34 <sup>###</sup>
Livopick (100mg/kg) (LD)	112.36±4.56 <sup>###</sup>	190.45±2.87 <sup>###</sup>	456.13±5.76 <sup>###</sup>

Values are expressed as MEAN±SEM. n=6. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  when compared to normal. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  when compared with toxic control.

Table 2: Effect of Livopick on serum bilirubin (total and direct) in PCM induced liver toxicity in rats.

Treatment	Total bilirubin (Mg/dl)	Direct bilirubin (Mg/dl)
Normal control	0.98±0.080	0.35±0.090
Toxic control (PCM)	3.78±0.450 <sup>***</sup>	1.56±0.050 <sup>***</sup>
Silymarin (100mg/kg) (STD)	1.24±0.540 <sup>###</sup>	0.50±0.120 <sup>###</sup>
Livopick (150mg/kg) (HD)	1.34±0.230 <sup>###</sup>	0.68±0.110 <sup>###</sup>
Livopick (100mg/kg) (LD)	1.50±0.340 <sup>###</sup>	0.75±0.230 <sup>###</sup>

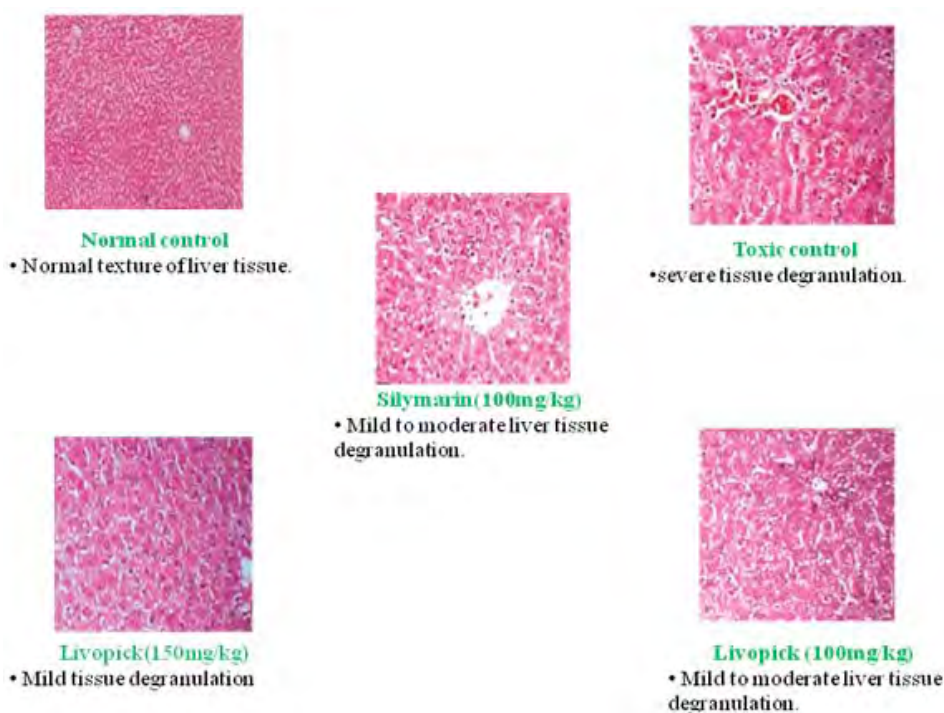
Values are expressed as MEAN±SEM. n=6. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  when compared to normal. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  when compared with toxic control.

Table 3: The effect of the Livopick on antioxidants against paracetamol induced liver toxicity in rats.

Treatment	GSH U/Mg	CATALASE U/Mg	SOD U/Mg
Normal control	4.78±0.02	73.45±0.12	8.23±0.22
Toxic control (PCT)	3.19±0.04 <sup>***</sup>	36.63±0.14 <sup>***</sup>	4.65±0.11 <sup>***</sup>
Silymarin (100mg/kg) (STD)	4.67±0.10 <sup>###</sup>	68.34±0.09 <sup>###</sup>	7.73±0.77 <sup>##</sup>
Livopick (150mg/kg) (HD)	3.17±0.07 <sup>###</sup>	66.98±0.21 <sup>###</sup>	7.96±0.63 <sup>##</sup>
Livopick (100mg/kg) (LD)	3.55±0.09 <sup>##</sup>	66.45±0.05 <sup>###</sup>	7.65±0.66 <sup>##</sup>

Values are expressed as MEAN±SEM. n=6. \*p <0.05, \*\*p < 0.01, \*\*\*p<0.001 when compared to normal. #p <0.05, ##p < 0.01, ###p<0.001 when compared with toxic control.

#### Histopathological studies:



Haematoxylin and eosin (H&E) stained section of liver in paracetamol induced liver toxicity, photographed at magnification 100X.

#### DISCUSSION:

The hepatoprotective effect of polyherbal formulation livopick in rats were studied during hepatic damages induced by paracetamol.

Paracetamol is normally eliminated mainly as sulfate and glucuronide. Upon administration of toxic doses of paracetamol the sulfation and glucuronidation routes become saturated and hence, higher percentage of paracetamol molecules is oxidized to highly reactive N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome-450 enzymes. Semi Quinone a radical obtained by one electron reduction of NAPQI, can covalently bind to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently, leads to increased lipid peroxidation and liver damage. Significant hepatic damage due to paracetamol is evident from the fact that there is elevation in the levels of various biochemical markers like ALT, AST, ALP, and bilirubin. Decreased level of enzymatic antioxidants is a clear manifestation of excessive formation of free radical during the metabolism of the paracetamol and activation of lipid peroxidation system.<sup>15</sup>

In this model paracetamol 2 g/kg (*i,p*) caused hepatotoxicity as indicated by the elevation of biochemical markers like SGPT, SGOT, ALP, bilirubin (total and direct) and decreased antioxidants enzymes such as GSH, catalase, SOD. In addition paracetamol administration disrupted the liver cells. Treatment with livopick reversed the elevated levels of all the biochemical markers and decreased antioxidant enzyme to the near normal levels in this model. It also reversed antioxidants activity which was decreased in the toxic. This enhanced hepatoprotective effect was also supported by histopathological report.

Hence, the possible mechanism behind the hepatoprotection may be of antioxidant effect, inhibition of lipid peroxidation chain reaction, free radical scavenging activity of herbal constituents or its synergistic effect in the formulation.

### CONCLUSION

With the findings of the present study it can be concluded that the polyherbal formulation livopick has significant increase in hepatoprotective effect against paracetamol induced hepatotoxicity in rats.

It is worth mentioning that livopick efficiently trims down the elevated levels of blood serum biomarkers, and also restored antioxidant parameters without producing any adverse effect. The results from the present study and histological analysis indicate the administration of livopick has protective effects against paracetamol induced hepatic necrosis state. These finding strengthen the observation that naturally occurring compounds of plant origin are effective in liver diseases.

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