

# Hepatoprotective Effect of A Polyherbal Extract Containing *Andrographis Paniculata*, *Tinospora Cordifolia* and *Solanum Nigrum* Against Paracetamol Induced Hepatotoxicity

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## ABSTRACT

**Background:** Traditionally, a number of medicinal plants are used to treat various types of hepatic disorders but few of them were pharmacologically evaluated for their safety and efficacy. The combination of *Andrographis paniculata* (*Kalmegha*), *Tinospora cordifolia* (*Guduchi*), and *Solanum nigrum* (*Kakmachi*) was traditionally used in Indian System of Medicine (*Ayurveda*) for the treatment of various liver-related disorders. **Objective:** In the present study, an attempt was made to substantiate the ethnopharmacological use of a traditional formulation in hepatoprotection against paracetamol-induced hepatotoxicity. **Subjects and Methods:** Swiss albino mice (weight 20–25 g) were used for this study. Intraperitoneal injection of paracetamol (500 mg/kg body weight) was used to induce hepatotoxicity. Serum levels of alanine transaminase, aspartate aminotransferase, bilirubin, alkaline phosphatase, were used as indices of liver injury. In addition total cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein and creatinine were also assayed using the standard procedure. **Results:** Among the two different doses, pretreatment with Polyherbal extract at 500 mg/kg body weight exhibited a significant ( $P < 0.05$ ) hepatoprotective activity as compared to paracetamol group. **Conclusion:** The polyherbal extract exhibits a significant hepatoprotective effect *in vivo*. The study contributes to its use in traditional *ayurveda* system for the management of liver diseases.

**Key words:** *Andrographis paniculata*, *Ayurveda*, hepatoprotective, paracetamol, *Solanum nigrum*, *Tinospora cordifolia*

## SUMMARY

Traditionally, a number of medicinal plants are used to treat various types of liver disorders but few of them were pharmacologically evaluated for their safety and efficacy. Combination of *Andrographis paniculata* (*Kalmegha*), *Tinospora cordifolia* (*Guduchi*), and *Solanum nigrum* (*Kakmachi*) was traditionally used in *Ayurveda* for the treatment of various liver related disorders. In the present study an attempt was made to validate the ethnopharmacological use of a traditional formulation in hepatoprotection against paracetamol induced hepatotoxicity. Swiss albino mice (weight 20-25 g) were used for this study. Intraperitoneal injection (IP) of paracetamol (500 mg/kg body weight) was used to induce hepatotoxicity. Serum levels of Alanine transaminase (ALT), Aspartate Aminotransferase (AST), Bilirubin, Alkaline phosphatase (ALP),

were used as indices of liver injury. In addition total cholesterol, triglyceride, Low density lipoprotein (LDL), High density lipoprotein (HDL) and creatinine were also assayed using standard procedure. Among the two different doses, pre-treatment with Polyherbal extract at 500 mg/kg body weight exhibited a significant ( $P < 0.05$ ) hepatoprotective activity as compared to paracetamol group. The polyherbal extract exhibits significant hepatoprotective effect *in vivo*. The study contributes to its use in traditional *Ayurveda* system for the management of liver diseases.



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## INTRODUCTION

As per an estimate by WHO the prevalence of liver diseases is expected to increase and contribute as a major factor for morbidity and mortality.<sup>[1,2]</sup> Being a principal site of metabolism and excretion, liver is involved in most of the biochemical pathways to combat disease, supply nutrient and energy to the cell. Drug-induced hepatotoxicity is one of the challenging clinical problems that account for 13% of all cases of the acute liver failure.<sup>[3]</sup> Many commonly known drugs viz. acetaminophen, allopurinol, rifampin, risperidone, statins, etc., when misused, can lead to hepatotoxicity.<sup>[4]</sup> Out of which, the paracetamol is responsible for the majority of cases when administered at a dose  $\geq 1000$  mg.<sup>[5-7]</sup> Liver is an important organ in the human body that offers the first line of protection against damage by ingested agents therefore, to live a hale and hearty life it is very important to protect the liver.

Traditionally, a number of medicinal plants like *Silybum marianum*, *Andrographis paniculata*, *Picrorhiza kurroa*, *Boerhavia diffusa* are used to treat various types of hepatic disorders but few of them are

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pharmacologically evaluated for their safety and efficacy.<sup>[8,9]</sup> According to the *Ayurveda*, the traditional Indian system of medicine, plants are composed of a mixture of compounds and when plants with common desired effects are mixed together some of the compounds present in them increase the therapeutic effect while others counteract the possible side effect that may exist.<sup>[10]</sup> In this viewpoint, the present study was undertaken to investigate the hepatoprotective activity of a polyherbal extract containing three well-known medicinal plants *A. paniculata* (*Kalmegha*), *Tinospora cordifolia* (*Guduchi*), and *Solanum nigrum* (*Kakmachi*) in a preset ratio. These plants have been used in the treatment and management of a number of ailments for, e.g. *A. paniculata* is reported for hepatoprotective, immunomodulatory and anti-cancer activity,<sup>[11]</sup> *T. cordifolia* for anti-inflammatory, anti-arthritis and hepatoprotective<sup>[12]</sup> and *S. nigrum* for anti-proliferative, anti-inflammatory, anti-seizure and hepatoprotective activity.<sup>[13]</sup> However, no reports are available to provide empirical data for their use as a hepatoprotective formulation in a combination of these plants.

Hence, the present study was undertaken to evaluate the hepatoprotective effect of this polyherbal extract in paracetamol-induced liver toxicity in Swiss albino mice.

## SUBJECTS AND METHODS

### Medicines and chemicals

Paracetamol pure salt was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl alcohol, potassium dihydrogen phosphate, dibasic monohydrogen phosphate, formaldehyde, paraffin wax, xylene, hematoxylin and the biochemical kits were purchased from Merck Chemicals Private Ltd. India. Liv-52 was procured from Himalaya Herbal Healthcare Ltd, India. All the chemicals and solvents used in this study were of analytical grade.

### Plant material

Plant materials were collected from the farm of CSIR-CIMAP, Lucknow and authenticated by Dr. Subhash Singh in Department of Botany, CSIR-CIMAP, Lucknow, India. A voucher specimen of *A. paniculata* (12,566), *T. cordifolia* (12,569) and *S. nigrum* (12,567) were preserved in the herbarium of CSIR-CIMAP, Lucknow, UP, India.

### Preparation of the extract

The aerial parts of *A. paniculata*, stem of *T. cordifolia* and fruits of *S. nigrum* were collected, dried in shade and powdered using a mill. The powders were mixed in a defined ratio of 2:1:1 and extracted with ethanol:water (1:1) at 60–70°C using soxhlet apparatus. 100 g of plant material was taken, and 400 ml of solvent was added to it for extraction. The extract was concentrated in a rotary evaporator (Buchi R-210, Switzerland). It was freeze-dried under vacuum over 50 h using the Lobconco freeze drying System (–42––47°C temperature with 0.340 mbar pressure). Once the material was completely dried, it was collected, placed in a desiccator at room temperature until use.

### Experimental animals

Swiss albino mice (weight 20 ± 5 g) were maintained in an air-conditioned room (22 ± 3°C); with relative humidity 50 ± 5%; and 12 h light/12 h dark cycle and acclimatized for 1-week. They were fed with certified pellet rodent diet (Dayal Industries, Lucknow, India) and water was provided *ad libitum*. Institutional Animal Ethics Committee of CSIR-CIMAP approved the protocol of the experiment (AH-2012-10). The guidelines for animal care were followed as recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

## Paracetamol induced hepatotoxicity in Swiss albino mice

The mice were divided into five groups of six animals each. Vehicle control (Group 1) and paracetamol group (Group 2) were administered with distilled water. Positive control (Group 3) was administered with Liv-52 (5.2 ml/kg) once daily for 7 days.<sup>[14]</sup> In the two experimental groups (Group 4 and 5), the mice were pretreated orally with polyherbal extract (300 mg/kg body weight, 500 mg/kg body weight) once daily for 7 days. One hour after, the last administration, all the groups except vehicle control were treated with paracetamol 500 mg/kg body weight in distilled water, intraperitoneally (IP).<sup>[8,14]</sup> After 2:30 h of paracetamol administration, 500 µl of blood was collected from the retro-orbital plexus of the mice using nonheparinized hematocrit capillaries. The blood was allowed to clot at room temperature for 30 min and centrifuged at 2500 rpm at 4°C for 10 min. The serum was collected and stored at –20°C for experimental analysis.<sup>[15,14]</sup>

### Histopathological studies

After blood collection, animals were sacrificed by cervical dislocation; the liver was dissected out and washed with phosphate buffer (pH 7.4) for complete removal of blood stains and clot. After washing, tissue was fixed in 10% formalin and embedded in molten paraffin wax followed by cutting of sections with a microtome. Deparaffinized sections were then stained with hematoxylin and eosin and observed microscopically for histopathological changes.

### Biochemical analysis

The collected blood samples were used for the analysis of different biochemical parameters viz. aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin, total cholesterol, triglyceride, low-density lipoprotein (LDL), high density lipoprotein (HDL) and creatinine by using standard diagnostic kits as per manufacturer's protocol (Lab kit from Merck Chemicals Private Ltd).

### Statistical analysis

Analysis of variance followed by Student–Newman–Keuls test was done using Instant GraphPad (version 3.0).  $P < 0.05$  was considered significant, and the values shown in tables are mean ± standard error of the mean (SEM).

## RESULTS

In the present study, the IP injection of paracetamol caused significant ( $P < 0.05$ ) hepatocellular changes as evident from enhanced levels of AST, ALT, ALP, and bilirubin compared to normal values. Pretreatment with polyherbal extract at 500 mg/kg body weight dose exhibited a significant ( $P < 0.05$ ) protection of liver function tests, similar to the results found during pretreatment with Liv-52 (a popular liver tonic) [Table 1].

Hepatic injury caused by paracetamol at a dose of 500 mg/kg body weight showed significant increase in the lipid profile, viz., total cholesterol, LDL, and triglycerides levels, whereas HDL level was decreased as compared to that of control group ( $P < 0.05$ ). However, pretreatment with polyherbal extract at a dose of 500 mg/kg body weight to paracetamol-induced group shows a significant decrease ( $P < 0.05$ ) in serum levels of triglyceride and LDL. The efficacy of the polyherbal extract was not found significant in the case of total cholesterol, but HDL level was improved significantly ( $P < 0.05$ ) as compared to paracetamol group [Table 1]. In this study the 300 mg/kg body weight dose of polyherbal extract shows significant ( $P < 0.05$ ) effect only on bilirubin and HDL but another biochemical parameter does not exhibit any

significant effect when compare to paracetamol affected group [Table 1]. Administration of paracetamol at dose 500 mg/kg body weight did not show any significant change in normal serum creatinine level [Table 1]. Histopathological analysis demonstrated a degeneration of normal architecture of liver cells, infiltration of the lymphocytes, loss of cell boundaries and the collapse of epithelial tissues (pointed through arrows) caused by the paracetamol. In the present study the mice pretreated with Liv-52 syrup and polyherbal extract at 500 mg/kg body weight dose, offered less damage compared to paracetamol group. Pretreatment with polyherbal extract at the dose 300 mg/kg body weight did not show any protective effect [Figure 1].

## DISCUSSION

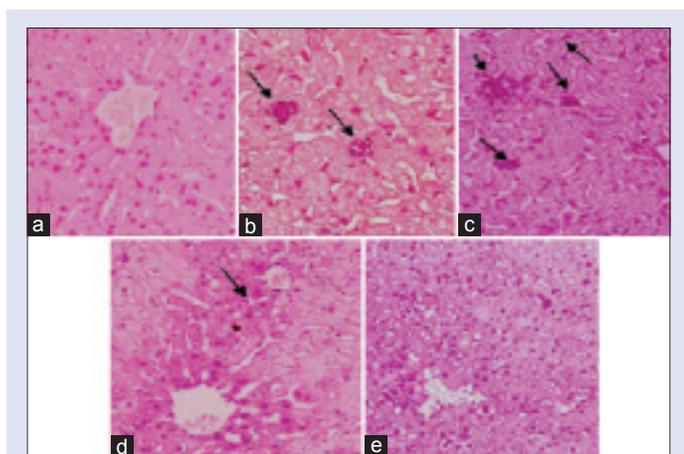
Acute liver failure is one of the leading problems worldwide. Hepatic adverse events caused by well-known drugs are of major concern among physician and health care professionals. Medicinal plants either in their isolated form or in the compound formulation have drawn attention all around the world for the treatment of various liver diseases. In *Ayurveda*, the compound formulations are generally used to enhance the therapeutic effect of individual plants and reduce the side effect if there are any.<sup>[16]</sup>

Paracetamol, a widely used analgesic, and anti-pyretic drug, is considered to be safe and nontoxic at prescribed doses. However, at repeated and high doses it becomes a potent hepatotoxic. Paracetamol induced liver toxicity is the leading cause of acute liver failure.<sup>[17]</sup> At higher dose

paracetamol mediated acute liver failure is initiated by production of certain reactive metabolites N-acetyl-p-benzoquinone imine generated by several hepatic cytochrome P-450 isoenzymes.<sup>[18,19]</sup> In liver injury, the transport mechanism of hepatocytes gets disturbed, resulting in the leakage of the plasma membrane, thereby causing an increased enzyme level in serum.<sup>[20]</sup> Administration of paracetamol (500 mg/kg body weight) significantly raises the serum level of ALT and AST in mice which was considered as a predictor of liver injury.<sup>[21-23]</sup> Oral administration of the polyherbal extract at a dose of 500 mg/kg body weight seems to protect the possible hepatic tissue damage caused by paracetamol as it reduces the serum levels of AST, ALT. An overdose of paracetamol causes depletion of glutathione and excessive metabolites reacts with the liver macromolecules and cause hepatic cell death leading to an elevated level of hepatic cellular enzyme ALP in serum.<sup>[24]</sup> Reduction of elevated bilirubin and ALP level was observed in the serum of mice pretreated with polyherbal extract 500 mg/kg body weight and Liv-52 with paracetamol.

In addition, total cholesterol, LDL, and triglyceride level increases in serum while HDL level decreased in paracetamol treated animals. Elevated levels of total cholesterol and triglyceride may be due to cholesterolemia and modest triglyceridemia, a condition commonly occurs in hepatocellular diseases as described by McIntyre and Glickman *et al.*<sup>[25,26]</sup> The animals pretreated with polyherbal extract (500 mg/kg body weight) prevented the increase of triglycerides in serum, which shows its protective nature against paracetamol toxicity but it has no effect on serum cholesterol. It has been reported that, hypolipidemic drugs with antioxidant properties, may prevent LDL peroxidation and retard their accumulation.<sup>[14,27,28]</sup> The decreased levels of serum HDL in the paracetamol treated mice may be due to free radicals produced during biotransformation. Mice pretreated with the polyherbal extract showed improved levels of HDL, which may be due to the ability of the extract to accelerate the decomposition of free radical species generated during acetaminophen toxicity.

Hepatoprotective effect of the polyherbal extract was further confirmed by the histopathological study of the liver sections, which supported the results obtained from the serum biochemical assays. Histopathological study of the liver tissues showed fatty changes, swelling with the loss of hepatocytes, centrilobular necrosis with lymphocytes and Kupffer cells infiltration in paracetamol intoxicated mice's. Animals in the group pretreated with polyherbal extract (500 mg/kg body weight) showed regeneration of hepatocytes, normalization of fatty changes and necrosis. The histopathological observations of the liver tissues of mice pretreated with the polyherbal extract showed more or less normal architecture of the liver comparable to the vehicle control group. Furthermore, the obstruction in lymphocytes and Kupffer cell penetration-induced by paracetamol



**Figure 1:** (a) Group 1 (vehicle control) (b) Group 2 (paracetamol) (c) Group 3 (Liv-52), (d and e) Group 4 and 5 hydro-alcoholic polyherbal extract at 300 mg/kg and 500 mg/kg body weight

**Table 1:** Effect of polyherbal extract on biomarkers for assessing hepatotoxicity in Swiss albino mice

Biochemical marker	Group 1 (vehicle control)	Group 2 (paracetamol)	Group 3 (Liv-52 + paracetamol)	Group 4 (PHE 300 mg/kg + paracetamol)	Group 5 (PHE 500 mg/kg + paracetamol)
AST (IU/L)	24.27±2.20	55.28±3.32***	30.275±1.61**	48.15±2.07	34.33±1.65**
ALT (IU/L)	32.50±1.22	63.59±3.36***	38.69±1.17**	54.08±3.06	41.85±1.92**
ALP (IU/L)	190.73±12.75	303.01±12.19***	231.05±17.99**	273.08±9.80	240.54±3.08**
Bilirubin (mg/dL)	0.40±0.10	2.40±0.19***	1.32±0.10**	1.9±0.74*	1.46±0.09**
Total cholesterol (mg/dL)	156.09±5.49	262.35±7.59***	221.81±6.97*	236.38±8.01	231.08±4.91
LDL (mg/dL)	77.77±3.76	122.53±4.21***	97.92±4.40*	116.89±5.62	102.97±2.05**
HDL (mg/dL)	33.90±1.62	20.67±1.61**	31.06±2.33*	27.37±1.90*	33.68±2.44**
Triglycerides (mg/dL)	74.42±3.84	133.35±6.13***	100.67±3.51*	114.95±3.75	92.07±3.88**
Creatinine (mg/dL)	0.57±0.043	0.70±0.043	0.65±0.052	0.64±0.047	0.66±0.041

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ . Values are expressed as mean±SEM ( $n=6$ ), Group 2 (paracetamol) was compared with group 1 (vehicle control). Group 3, 4 and 5 (Liv-52 and treatment groups) were compared with group 2 (paracetamol). AST: Aspartate Aminotransferase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; SEM: Standard error of the mean; PHE: Polyherbal extract

was significantly decreased by polyherbal extract (500 mg/kg body weight) indicating its hepatoprotective action. Additional research related to this polyherbal extract is warranted, to explore the exact mechanism and the constituents responsible for the pharmacological activity.

## CONCLUSION

The present work analyzed the hepatoprotective potential of an *Ayurvedic* polyherbal extract containing *A. paniculata*, *T. cordifolia* and *S. nigrum* in the ratio of 2:1:1. Accordingly, the polyherbal extract could be developed as an effective hepatoprotective agent in the management of liver ailments, as well as a lipid profile.

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## Conflicts of interest

There are no conflicts of interest.

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