

Protective effect of *Triphala Rasayana* against paracetamol-induced hepato–renal toxicity in mice

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ABSTRACT

Background: Paracetamol, a widely used analgesic and antipyretic, is known to cause liver and renal injury in humans when administered in higher and repeated doses that cause acute liver injury. *Triphala* is a well-known *Ayurvedic Rasayana* formulation that is prescribed for balancing of *Vata*, *Pitta* and *Kapha*. Traditionally, it is used for the treatment of liver and kidney diseases. **Objective:** The present study was undertaken to examine the protective effect of *Triphala* extract against paracetamol-induced hepato–renal injury in Swiss albino mice. **Materials and Methods:** Swiss albino mice (weight 20–25 g) were used in this study. The mice were divided into five groups of six animals each. The aqueous extract of *Triphala* was given orally at two different doses (100 and 300 mg/kg body weight) for seven consecutive days, followed by a single intraperitoneal injection of paracetamol (500 mg/kg body weight) to induce hepato–renal toxicity. Serum levels of liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, creatinine, urea and uric acid were measured as indices of liver and renal injury. All the statistical analyses were performed with the help of one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test as *post hoc* test. Results were considered statistically significant when $P < 0.05$. **Results:** Pre-treatment with *Triphala* extract at 100 mg/kg and 300 mg/kg body weight exhibited a significant ($P < 0.01$) hepatoprotective activity. The protective effect of *Triphala* extract at 300 mg/kg body weight appears more effective than 100 mg/kg body weight. **Conclusion:** The present study gives an evidence of the protective role of *Triphala* extract against paracetamol-induced hepato–renal toxicity and validates its traditional claim in the *Ayurveda* system.

Key words: *Ayurveda*, kidney, liver, paracetamol, *Triphala*

INTRODUCTION

Paracetamol belongs to the class of medications called analgesic (pain reliever) and antipyretic (fever reducer) and is widely used as an over-the-counter (OTC) pain and fever medication to relieve mild to moderate pain from

head, muscle, menstrual period, cold, sore throat, tooth, reactions to vaccination (shot), in osteoarthritis (arthritis caused by the breakdown of the lining of the joints) and to reduce fever. Paracetamol is known to cause liver and renal injury in humans and experimental animals when administered in higher and repeated doses. The incidences associated with liver injury include abdominal pain, nausea, vomiting, low blood sugar, low blood pH, easy bleeding, oliguria (decreased output of urine) and hepatic encephalopathy. The excess use of paracetamol leads to liver and renal failure, and even causes death.^[1]

The mechanism of paracetamol toxicity in the liver is well described, but in the kidney it is less clearly understood. Paracetamol is primarily metabolized and excreted as paracetamol–glucuronide and paracetamol–SO₄ in the hepatocytes. When paracetamol is taken at a higher dose (larger amounts of paracetamol are bioactivated), it produces a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), generated by several hepatic cytochrome P-450 isoenzymes.^[2] Glutathione (GSH) can reduce NAPQI, recycling to paracetamol, or conjugate the NAPQI

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forming paracetamol–GSH, which is excreted from the hepatocyte. Generation of the reactive oxygen species has appeared as an early event that is preceded by intracellular GSH depletion. Depletion of GSH level permits the protein thiolarylation by NAPQI that may compromise the protein function and contribute to paracetamol, leading to hepatocellular damage.^[3] Paracetamol-induced renal insufficiency depends on the drug, dose, duration of pharmacological effect and the health of the patient.^[4] Overdose of paracetamol causes various adverse effects in the kidney, which may partly be due to the vasoconstriction resulting in inhibition of renal prostaglandin-mediated vasodilatation, decreasing renal blood flow and finally decreasing the glomerular filtration rate.^[5] Oxidative stress, with decline of GSH and lipid peroxidation, has also been suggested as a mechanism by which paracetamol could induce renal injury.^[6]

Triphala is the combination (equal proportion 1:1:1) of dried fruits (Phala) of *Amalaki* (*Embelica officinalis*), *Bibhitaki* (*Terminalia bellirica*) and *Haritaki* (*Terminalia chebula*). As per *Ayurveda*, in the Indian system of medicine, it is commonly used to balance the three *doshas* (humors) in the body, viz. *Vatta*, *Pitta* and *Kapha*.^[7] Traditionally, the *Triphala* formulation has been used as a laxative in chronic constipation, as a detoxifying agent of the colon, and as a rejuvenator of the body.^[8] According to recent researches, the *Triphala* formulation also shows antidiabetic^[9] and hepatoprotective activities and plays an important role in blood pressure control and balances cholesterol.^[10] *Triphala* is an important medicine in *Rasayana* therapy that preserves and promotes health, immunity and longevity.^[11] The individual plants of *Triphala* have been reported to be a rich source of Vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, β -sitosterol and flavanoids,^[12] which possess several other health benefits as well. These herbs are also reported for their anti-inflammatory, anti-mutagenic, antioxidant, cytoprotective, gastroprotective, anti-cancer activity and anti-bacterial^[10] effects. The protective effect of *Triphala* extract against paracetamol-induced hepato–renal toxicity has not yet been reported till date to the best of our knowledge. This study was, therefore, undertaken to examine the protective effect of *Triphala* against paracetamol-induced hepatic–renal toxicity in Swiss albino mice.

MATERIALS AND METHODS

Drugs and chemicals

Paracetamol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium dihydrogen phosphate, dibasic monohydrogen phosphate, formaldehyde, paraffin wax, xylene and biochemical kits were purchased from Merck

Chemicals Private Ltd. Shive nagar, Mumbai, India. Liv-52 was procured from Himalaya Herbal Healthcare Ltd. Makali, Tumkur Rd, Bengaluru, Karnataka after 2 months of manufacture. All the chemicals and solvents used in the study were of analytical grade.

Plant materials

Dried fruits of *Amalaki* (*Embelica officinalis*), *Bibhitaki* (*Terminalia bellirica*) and *Haritaki* (*Terminalia chebula*) were collected from the farm of CSIR-CIMAP, Lucknow and authenticated by an *Ayurvedic* physician at the HMPD, CSIR-CIMAP Lucknow, India. A voucher specimen of *Amalaki* (*Embelica officinalis*) (HMP101), *Bibhitaki* (*Terminalia bellirica*) (HMP102) and *Haritaki* (*Terminalia chebula*) (HMP103) was deposited in the departmental herbarium of the CSIR-CIMAP, Lucknow, UP, India.

Preparation of the extract

Dried fruits of *Amalaki*, *Bibhitaki* and *Haritaki* were collected and powdered using a mill. The dried powder of all the three fruits was mixed in a defined ratio of 1:1:1 and the mixture was extracted with distilled water by using a cold maceration process. One hundred grams of the above mixture was taken and soaked overnight in 400 mL of distilled water. The extract was filtered using a filter paper (Whatman filter paper grade 1) and then the extract was concentrated in a rotary evaporator (Buchi R-210, Switzerland) and freeze-dried under vacuum for 50 h using the Lobconco freeze drying system (-42°C to -47°C temperature with 0.340 mbar pressure). Completely dried material was collected and stored in a closed air-tight container at room temperature until use.

Experimental animals

Swiss albino mice (weight 20–25 g) were maintained in an air conditioned room ($22 \pm 3^{\circ}\text{C}$) with relative humidity of $50 \pm 5\%$ and 12-h light/12-h dark cycle and acclimatized for 1 week prior to the study. They were fed with a certified pellet rodent diet (Dayal Industries, Lucknow, India) and water was provided *ad libitum*. The 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 (CPCSEA), Government of India.

Paracetamol-induced hepato–renal toxicity in mice

Paracetamol overdose was used to induce hepato–renal toxicity in Swiss albino mice. The mice were divided into five groups of six animals each.

Group 1: (Vehicle control) Animals received distilled water for 7 days and served as normal controls

- Group 2: (Paracetamol) Animals in this group received (only the paracetamol challenge) distilled water
- Group 3: (Liv-52) Positive control group, pre-treated with Liv-52 at 5.2 mL/kg body weight (BW) for 7 days^[13]
- Group 4 and Group 5: Served as the treatment groups and pre-treated with *Triphala* extract at 100 mg/kg and 300 mg/kg body weight for 7 days.

On the 8th day, all the groups except Group 1 were given an intraperitoneal injection of paracetamol at 500 mg/kg BW. After 2.5 h of paracetamol administration, 500 µL of blood was collected from the retro-orbital plexus of the mice using non-heparinised hematocrit capillaries. The serum was separated and stored at -20°C for experimental analysis.^[14]

Biochemical analysis

The collected blood samples were used for the analysis of different biochemical parameters, *viz.* aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Bilirubin, Creatinine, Urea and Uric acid, by using standard diagnostic kits as per the manufacturer's protocol (Labkit from Merck Chemicals Private Ltd., India).

Histopathological studies

After blood collection, animals were sacrificed and their 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 microtome. Deparaffinized sections were then stained with hematoxylin and eosin and observed microscopically for histopathological changes.

Statistical analysis

All the statistical analyses were performed with the help of Graph Pad InStat software (version 3.0). Difference between means was calculated by one-way analysis of

variance (ANOVA) followed by the Student–Newman–Keuls test as a *post hoc* test. $n = 6$ mice/group. Results were considered statistically significant when $P < 0.05$.

RESULTS

Effects of *Triphala* on liver and kidney functions

Intraperitoneal (IP) administration of paracetamol in Swiss albino mice (Group 2) at the dose of 500 mg/kg BW produced a significant ($P < 0.001$) increase in the serum levels of AST, ALT, ALP and Bilirubin, while pre-treatment with the Liv-52 syrup (5.2 mL/kg body weight) (Group 3) and *Triphala* extract at the rate of 100 mg/kg and 300 mg/kg BW (Groups 3 and 4) significantly ($P < 0.01$) prevented the elevation in these liver enzymes [Table 1, Figure 1]. Similarly, the serum levels of Creatinine, urea and uric acid were increased after paracetamol administration, which were significantly ($P < 0.001$) different from the vehicle control group. Pre-treatment with the *Triphala* extract at the above-mentioned dose and Liv-52 syrup effectively ($P < 0.01$) prevented the increase in the serum levels of these renal function tests [Table 1, Figure 1]. The effect of *Triphala* extract on liver function tests (AST, ALT, ALP and Bilirubin) and renal function tests (Creatinine, urea and uric acid) shows its effect in a dose-dependent manner. The *Triphala* extract exhibited more protective effects at 300 mg/kg BW than 100 mg/kg BW [Table 1 and Figure 1].

Effect of *Triphala* on histopathological changes

Histopathological observation of liver tissues showed that there were no abnormal morphological changes in the liver specimens of the control group [Figure 2a]. The liver samples of the paracetamol group showed necrosis, phagocyte filtration and fat degeneration [Figure 2b]. The liver tissues in the groups pre-treated with Liv-52 syrup and *Triphala* extract protects the liver cells, central vein and portal vein from paracetamol toxicity and maintains the normal architecture of the liver tissues [Figure 2c–e].

Table 1: Effect of *Triphala Rasayana* on liver and kidney bio-markers for assessing hepatic-renal toxicity in Swiss albino mice

Biochemi-cal marker	Group 1 (vehicle control)	Group 2 (paracetamol)	Group 3 (Liv-52)	Group 4 (PHE 100 mg/kg)	Group 5 (PHE 300 mg/kg)
AST (IU/L)	23.77±2.166	94.28±4.362***	59.71±3.906###	72.82±5.915##	65.99±2.868###
ALT (IU/L)	30.84±2.108	74.26±6.293***	48.43±4.348##	59.08±5.349#	50.51±3.227##
ALP (IU/L)	176.9±12.291	270.01±15.066***	200.06±13.325##	231.41±11.87	210.54±14.911#
Bilirubin (mg/dL)	0.405±0.1	2.422±0.35***	0.99±0.15###	1.58±0.23#	1.19±0.13##
Creatinine (mg/dL)	0.527±0.11	2.455±0.29***	1.0375±0.15###	1.55±0.16##	1.21±0.13###
Urea (mg/dL)	19.63±1.13	52.59±6.16***	29.77±3.33##	39.61±2.85#	34.02±3.97##
Uric acid (mg/dL)	0.576±0.079	1.83±0.27***	0.80±0.12##	1.47±0.17 ^{ns}	0.98±0.11##

Values are expressed as mean±SEM ($n=6$), Group 2 (Paracetamol) was compared with Group 1 (vehicle control), * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Groups 3, 4 and 5 (Liv-52 and treatment groups) were compared with Group 2 (Paracetamol). # $P \leq 0.05$, ## $P \leq 0.01$, ### $P \leq 0.001$, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphate, ^{ns}= Non significant

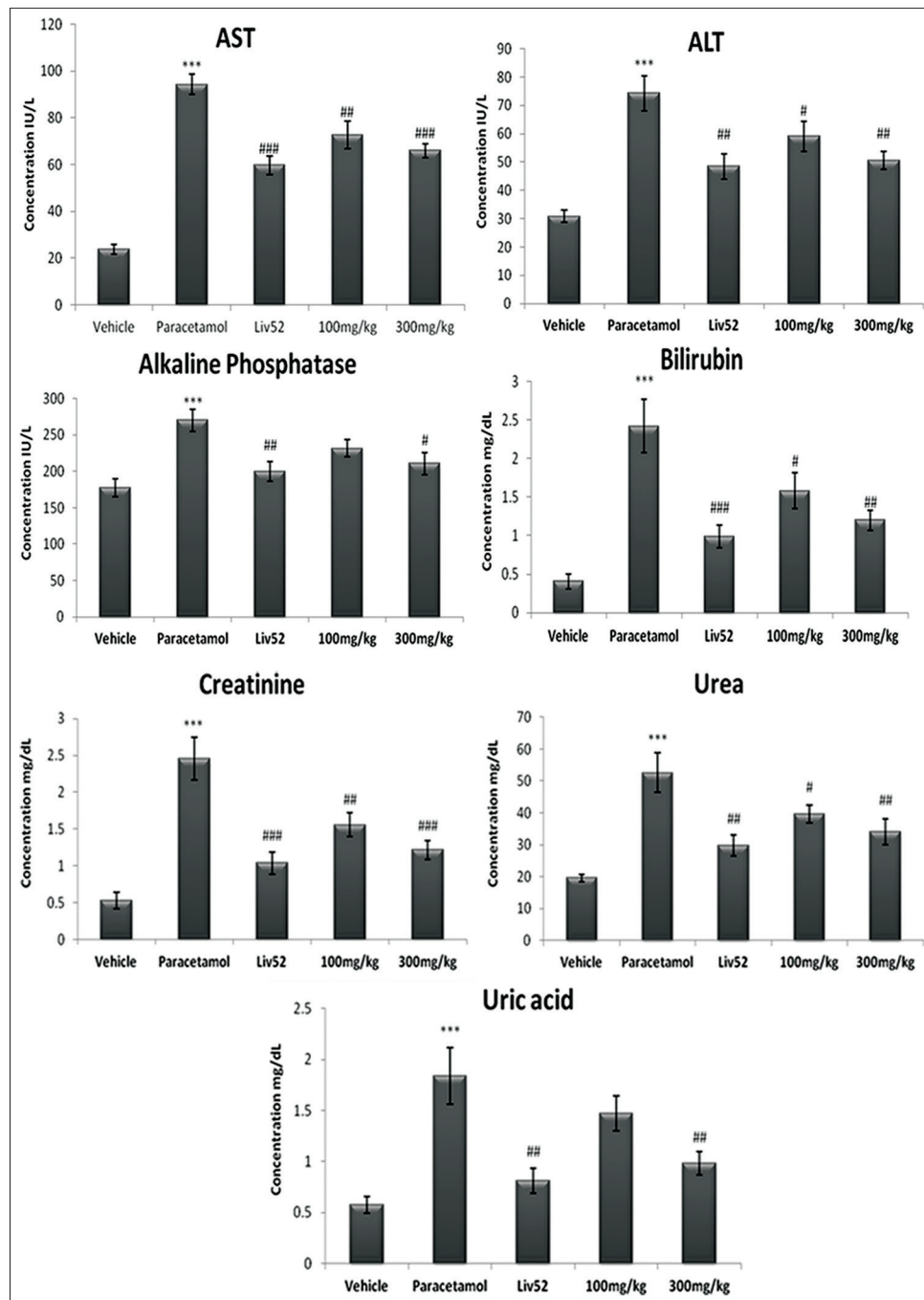


Figure 1: Effect of *Triphala* extract on different groups of mice against paracetamol-induced hepato–renal toxicity in mice. The effect of pre-treatment with *Triphala* extract at 100 mg/kg and 300 mg/kg body weight dose on AST, ALT, ALP, Bilirubin, Creatinine, urea and uric acid of mice in paracetamol-induced liver and renal toxicity. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, paracetamol compared with the vehicle control group, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, Liv-52 and treatment Groups 4 and 5 compared with the paracetamol group ($n = 6$ mice/group)

DISCUSSION

The liver is a multipurpose organ in the body related with regulation of various metabolic pathways. Therefore, damage of the liver tissues caused by a hepatotoxic agent is a serious consequence. Paracetamol is the most commonly used analgesic and antipyretic drug known to possess a hepatotoxic effect in humans as well as in animals at high doses.^[15] It is

generally observed that drug- or chemical-induced liver and kidney injuries are associated with the overproduction of free radicals.^[16,17] Parallel to this, renal insufficiency occurs in around 1–2% of patients with paracetamol overdose. Paracetamol-induced renal damage becomes obvious after hepatotoxicity in most of the cases.^[18] Thus, prevention and treatment of liver injury, together with renal protection, can be a good line of treatment in liver diseases.

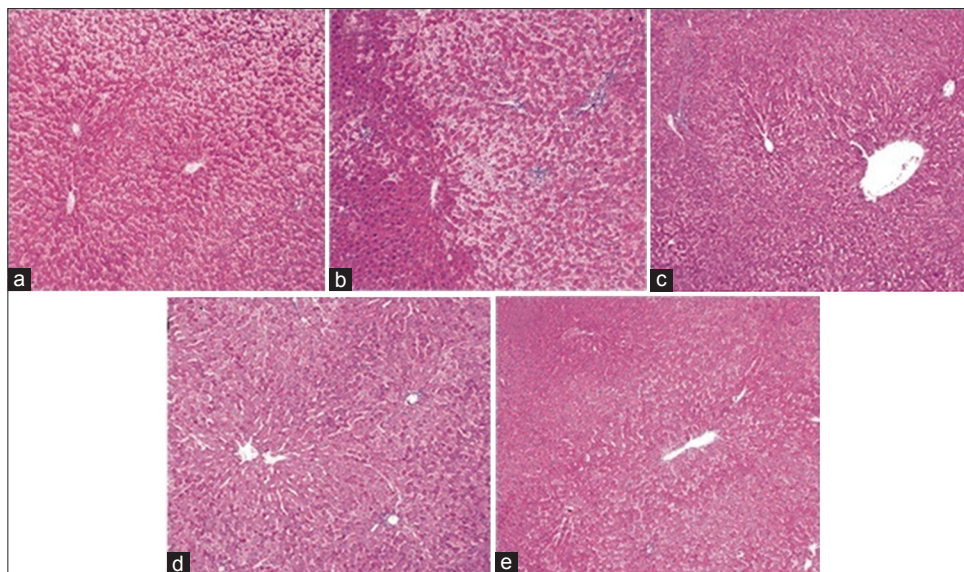


Figure 2: Histopathological features of the different groups of mice. Effect of *Triphala* extract on counteracting mice histopathological damages induced by paracetamol. Control group (a) mice showing normal histological architecture and chords of hepatocytes are radiating. Toxin (paracetamol)-induced group (b) showing centri-zonal necrotic areas and degenerated liver tissues. Liv-52 (5.2 mL/kg body weight) and *Triphala* extract at 100 mg/kg and 300 mg/kg body weight (c, d and e) showing protection of hepatocytes with mild changes and pyknotic nuclei around the central vein indicating necrotic changes

Herbal medicines have been used since antiquity and have played a pivotal role in the prevention and treatment of various diseases. A large number of herbs and herbal formulations are used in the Indian medicinal systems, viz. *Ayurveda*, *Siddha*, *Unani* and *Amchi*, etc., According to the World Health Organization (WHO), 80% of the people throughout the world currently use herbal medicines for primary health care, as well as most of the modern plant-derived pharmaceutical medicines are used in ways that correlated directly with their traditional uses.^[19] *Triphala* is considered as one of the most important *Rasayana* formulations in the Indian system of medicine (ISM). In this current research work, *Triphala* extract was screened for its possible hepato–renal protection in mice against paracetamol-induced toxicity.

In the present study, paracetamol-induced hepatotoxicity was evidenced by the biochemical measurements and histopathological changes, while renal damage was indicated by rise in serum levels of renal markers. The increased level of serum AST, ALT, ALP, Bilirubin, Creatinine, urea and uric acid in mice treated with paracetamol indicated altered liver and renal functions due to its toxic effects.^[20] Pre-treatment of mice with *Triphala* extract for seven consecutive days resulted in a significant protection of paracetamol-induced elevation of serum enzyme markers (AST, ALT, ALP, Bilirubin, Creatinine, urea and uric acid) given in two doses when compared with the paracetamol group [Figure 1]. The *Triphala* extract significantly reduced the elevated serum enzyme levels in the treatment group, which indicated

the ability of *Triphala* to preserve the normal functions of the liver and kidney. It can be proposed that *Triphala* inhibited the free radical generation and resultant damage to maintain the normal functions of the liver and renal cellular membrane; [Figure 1] although the exact mechanism of action needs further investigation. The hepatoprotective effect of the *Triphala* extract was further confirmed by histopathological studies of the liver tissues, which basically supported the results obtained from the serum assays. Moreover, as revealed by the histopathological study, the *Triphala* extract could provide protection against paracetamol-mediated hepatic alterations by membrane regeneration and, therefore, it may be helpful in limiting the drug-induced membranal damage in the liver and kidney. Furthermore, in our study, the results clearly show that the *Triphala* extract at the dose of 300 mg/kg BW offers more protection to liver tissues that were comparable to Liv-52, but in the case of 100 mg/kg BW, it offers comparatively less protection than Liv-52 against paracetamol-induced hepato–renal injury. However, a more elaborative study with special focus on the effects of *Triphala* in maintaining GSH levels and possible measurement of NAQPI using N ACEYL CYSTEINE as a positive control is still required.

CONCLUSION

In conclusion, the findings of the current study illustrated that the *Triphala* extract was effective in eliminating the paracetamol-induced hepato–renal injury. It may be a

potential lead for the protection of the liver and kidney against drug-induced toxicity.

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