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**Original Research Article**

# Effect on liver function of an ayurvedic medicine Punarnavasava after 28 days chronic toxicity studies on male Sprague Dawley rats

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## ABSTRACT

**Background:** Punarnavasava (PVR) is an ayurvedic formulation predominantly used as a traditional medicine in the rural population to get relief from edemic conditions. Though this medication is recognized as generally safe, clinical research on this formulation is scarce. Therefore, contributing to determining the safety of this drug may help ensure safer treatment for thousands of patients.
**Methods:** The effect of chronic administration of PVR on liver function was determined by administrating chronically to the male Sprague-Dawley rats at a dose of 40 ml per kg body weight for 28 days. In this experiment plasma protein, albumin, serum bilirubin and various serum enzymatic parameters were determined.
**Results:** In the study, the total protein content in the plasma was increased (11.05%) in the PVR treated male rats. The increase in total protein was not statistically significant but was noticeable (p=0.104). On contrary, the albumin content was decreased (7.92 %) in PVR treated male rats and it was not statistically significant, yet it was noticeable (p=0.076). No difference on the bilirubin level between the control and the experimental group was noted. In the PVR administered male rats, activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALK-P) was decreased by 31.90%, 45.44%, 82.82% and 32.53% respectively and all these changes were statistically very highly significant (p=0.0001) for all these enzymes. In contrast, a not significant negligible increment was observed in the GGT activity.
**Conclusions:** From this study, it is evident that there was no adverse increase in the liver function parameters in the male rats after chronic administration of PVR, on the contrary significant decrease in the serum liver function enzymes were noted, therefore, it can be concluded that this Asava preparation (PVR) can be administered chronically. With the slight increase in GGT though negligible suggests caution for use in pregnancy related edemic conditions which may deteriorate liver cholestasis related to later stage of pregnancy. Further research should be done to reconfirm the safety of this ayurvedic medicine in pregnancy.

**Keywords:** Ayurvedic Sandhan formulation, Punarnavasava, Asava, Liver function, Liver enzymes

## INTRODUCTION

Ayurvedic is one of the ancient Indian medical systems, based on ancient writings that rely on a 'natural' and holistic approach. This medicinal system is considered as one of the oldest treatment approaches that is still being used by millions of people worldwide. This traditional treatment combines natural products, diet, exercise and lifestyle. These medicines are generally effective and safe to treat various physical and mental conditions including pain, arthritis, skin diseases, cough, lung diseases, gastric, jaundice, spleen and liver diseases. Few well-designed clinical trials and systematic research reviews suggest that Ayurvedic approaches are effective in treating knee osteoarthritis, rheumatoid arthritis, type 2 diabetes and ulcerative colitis. However, some ayurvedic preparations

may contain metals and minerals which may make them potentially harmful.[1,2] Though these types of traditional treatment approaches are well accepted among the general population of rural areas because of their perception of lack of side effects and albeit safety, similar to natural products.[3] For many millions of people around the world, traditional medicine is the first port of call to treat many diseases.

Around 80% of the world's population, roughly around 6.5 billion, is estimated to use traditional medicines. Around 170 countries among 194 WHO member states have reported the use of traditional medicines.[4]

PVR is an Asava (Liquid) type ayurvedic formulation which has been indicated for deficiency of iron, ascites, dropsy and spleen disorders. In addition, it has diuretic and liver protecting properties. Herbs like Pippali, Shunti, Maricha, Darvi, Vibhitaki, Amla, Haritaki, Vasaka etc. are the major ingredients in the formulation of PVR (Table 1). It may contain 5-10% alcohol because it is produced by fermentation process.[5]

**Table 1: Name of the ingredients used in the preparation of PVR.[5]**

| Name | Botanical name | Part used | Quantity |
|---|---|---|---|
| **Shunti (Ginger)** | *Zingiber officinalis* | Rhizome | 48 gm |
| **Maricha (Pepper)** | *Piper nigrum* | Fruit | 48 gm |
| **Pippali (Long pepper)** | *Piper longum* | Fruit | 48 gm |
| **Haritaki** | *Terminalia chebula* | Fruit rind | 48 gm |
| **Vibhitaki** | *Terminalia bellirica* | Fruit rind | 48 gm |
| **Amla** | *Emblica officinalis* | Fruit rind | 48 gm |
| **Darvi** | *Berberis aristata* | Stem Wood | 48 gm |
| **Gokshura** | *Tribulus terrestris* | Fruit | 48 gm |
| **Brihati** | *Solanum indicum* | Root | 48 gm |
| **Kantakari** | *Solanum xanthocarpum* | Whole plant | 48 gm |
| **Vasaka** | *Adhatoda vasica* | Root | 48 gm |
| **Erandamoola (Castor)** | *Ricinus communis* | Root | 48 gm |
| **Katuki** | *Picrorhiza kurroa* | Root | 48 gm |
| **Gajapippali** | *Scindapsus officinalis* | Fruit | 48 gm |
| **Punarnava** | *Boerhavia diffusa* | Root | 48 gm |
| **Neem** | *Azadirachta indica* | Stem bark | 48 gm |
| **Guduchi** | *Tinospora cordifolia* | Stem | 48 gm |
| **Shushka Mulaka** | *Raphanus sativus* | Root | 48 gm |
| **Duralabha** | *Fagonia cretica* | Root | 48 gm |
| **Patola** | *Trichosanthes dioica* | Leaf | 48 gm |
| **Dhataki** | *Woodfordia fruticosa* | Flower | 768 gm |
| **Draksha (Dry grapes)** | *Vitis vinifera* | Fruit | 960 gm |
| **Sugar** | | | 4.800 kg |
| **Madhu (Honey)** | | | 2.400 kg |
| **Water** | | | 24.57 liters |

PVR is included (pages 145-146) in the Bangladesh national formulary of ayurvedic medicine 1992 which has been approved by the government of Bangladesh vide ministry of health and family welfare, memo no. Health-1/Unani-2/89/(Part-1) 116, dated June 3, 1991.

Liver is a vital organ, responsible for numerous functions which are very crucial for overall health, including detoxification, metabolism and immune support. Liver plays key role in metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects. Drug substances may be an important cause of hepatotoxicity. More than 900 drugs, toxins, and herbs have been reported to cause liver toxicity.[6]

Though complementary and alternative medicines are thought to be safe in general use, it has lack of evidence of safety. Contrary to popular belief, it is evident that apart from adverse events caused by contamination and adulteration of alternative medicines, certain commonly used herbal components have inherent hepatotoxicity.[7]

## METHODS

### *Drugs, chemicals and reagents*

For this toxicological research, the experimental Ayurvedic drug, PVR was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. ACI Pharmaceuticals Limited, Bangladesh has provided the Ketamine injection. All other reagents, assay kits and chemicals used in this research work were obtained from Human GmbH, Wiesbaden, Germany.

### *Experimental animals*

For this toxicological research work, healthy albino rats (*Rattus novergicus*: Sprague-Dawley strain,) of eight-week-old of both sexes were used. These animals weighed about 50-70 gm. The rats are maintained at the animal house of the Department of Pharmacy, Jahangirnagar University. Feeding of animals was done ad libitum, along with drinking water and maintained at natural day night cycle. The animals were housed in a well-ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. Rat chow which was prepared according to the formula developed by Bangladesh council of scientific and industrial research (BCSIR) was given as food for the selected research animals. Water was given ad libitum and the animals maintained at 12 hours day and 12 hours night cycle. All experiments on rats were carried out in absolute compliance with the Ethical guideline for care and use of laboratory animals approved by ethical review committee, faculty of life sciences, Department of Pharmacy, Jahangirnagar University.

### *Experimental design*

Prior to the experiment, male rats were randomly divided into 2 groups of 10 animals. Thus, ten rats were taken for each group for both control and experimental group. Among these two groups, the experimental group was administered with PVR, and control group was administered with distilled water as placebo, only as per the same volume as the experimental group gets for 28 days. For this study, the drug was administered per oral route at a dose of 40 mg/kg body weight and placebo (Distilled water) was given to the control group as same volume as experimental drug. After acclimatization, PVR preparation was administered to the rats by intra-gastric syringe between the 10 am and 12 noon daily throughout the study period.

### *Blood sample collection and preparation of plasma*

Completing the 28 days treatment, both animal groups kept fasting for 24 hours. The blood samples were collected from posterior vena cava of the rats anaesthetizing with ketamine (500 mg/kg body, intra peritoneal) and transferred into heparinized tubes immediately. Collected blood was centrifuged at 4,000 g for 10 minutes using top centrifuge (MSE Minor, England) to remove red blood cells and recover plasma. Plasma samples were separated and were collected using dry Pasteur pipette and stored in the refrigerator for analysis. All analyses were completed within 24 h of sample collection.

### *Determination of biochemical parameters*

Biochemical analysis was carried out on serum samples, to assess the state of the liver. Biochemical studies involved analysis of parameters such as total protein, serum albumin, bilirubin (total and direct), and liver enzymes such as ALT, AST, LDH, gamma glutamyl transferase (GGT) and ALK-P.

Total protein content of samples was assayed by Biuret method (Plummer, 1978).[8] Serum albumin concentration was determined using the method of Doumas et al.[9] The method of Malloy et al was employed to determine the serum bilirubin concentration of the samples.[10] ALT and AST activities were determined by the method of Reitman and Frankel (Bergmeyer and Bernt). LDH activities were determined using the method as described by Schumann et al.[11] GGT activities determined using method as described by Kaplan et al.[12] ALK-P activities were determined using the method as described by Abraham et al.[13] Absorbances of all the tests were determined using a spectrophotometer (UV-visible spectrophotometer model no. UV-1601 PC.).

### *Statistical analysis*

The data were analyzed using unpaired t-test as described by (Glasnapp et al) and expressed as mean±SEM (standard error of the mean).[14] SPSS for windows (ver-11) was applied for the analysis of data and $p<0.05$, $p<0.01$, $p<0.001$ was taken as the level of significance.

## RESULTS

In the study, the total protein content in the plasma was increased (11.05%) in the PVR treated male rats. The increase in total protein was not statistically significant but was noticeable ($p=0.104$). On contrary to the findings regarding total protein, the albumin content was decreased (7.92%) in PVR treated male rats and here also the decrease though not statistically significant yet it was noticeable ($p=0.076$). However, there was no change (0) found in the serum bilirubin level in the experimental animal after 28 days administration of PVR (Table 2).

**Table 2: Effect of PVR on serum protein and bilirubin level.**

| Parameters | Control | PVR | P values | % changes |
|---|---|---|---|---|
| | Mean±SEM | | | |
| **Total protein** | 44.12±2.16 | 49±1.78 | 0.104 | ↑11.05 |
| **Albumin** | 30±1.07 | 27.62±0.56 | 0.08 | ↓7.92 |
| **Bilirubin** | 0.34±0.05 | 0.34±0.03 | 1.00 | 0 |

*↑: increase, ↓: decrease

**Table 3: Effect of PVR on enzymatic activity in male rats.**

| Parameters | Control | PVR | P values | % changes |
|---|---|---|---|---|
| | Mean±SEM | | | |
| **ALT** | 82.86±4.07 | 56.43±1.96 | 0.001 | ↓31.90 |
| **AST** | 75.83±4.78 | 41.38±2.25 | 0.001 | ↓45.44 |
| **ALP** | 155.62±7.67 | 105.00±4.20 | 0.001 | ↓32.53 |
| **LDH** | 147.14±6.67 | 25.29±2.30 | 0.001 | ↓82.82 |
| **GGT** | 7.00±0.68 | 7.13±0.88 | 0.120 | ↑1.79 |

*↑: increase, ↓: decrease

In the male rats, ALT, AST, LDH and ALP activity decreased by 31.90%, 45.44%, 82.82% and 32.53% respectively and all these changes were statistically very highly significant (p=0.0001) for all these enzymes. On the contrary a negligible increase in sGGT activity (1.79%) was observed, which was not significant (p=0.12) (Table 3). From the study on ratio of different enzyme activities it was noted that change in AST was the most prominent, followed by the ALT and the GGT.

## DISCUSSION

Liver is a significant organ of the human body, responsible for transforming, metabolizing and cleansing chemicals or any xenobiotics. This organ gets the highest exposure to any foreign agents that is why it remains at risk of the toxicity from any chemicals, drugs or foreign particles. Certain medicinal agents in larger doses or even at therapeutic dosage may cause hepatotoxicity. In addition, chemical agents, natural chemicals (e.g., microcystins) and herbal medicines can also introduce hepatotoxicity which may ultimately cause liver failure.[15]

Adverse drug reactions are an important cause of liver injury that may require discontinuation of the offending agent, hospitalization, or even liver transplantation. Indeed, drug-induced hepatotoxicity is the most frequent cause of acute liver failure. Because the liver is responsible for concentrating and metabolizing most medications, it is a prime target for medication-induced damage.[16] The liver is an exceptional organ which protects an individual from injury caused by xenobiotic compounds. As liver acts as a metabolism site where chemical compounds tends to accumulate and bioactivate here, it is more susceptible to chemical injury.[17]

Serum protein encompasses a variety of proteins found in blood serum, crucial for assessing health and disease states. Serum protein is a collective term for blood proteins, including enzymes, hormones, and immunoglobulins, indicative of the body's biochemical status and overall health, as highlighted by regional sources. Total serum protein means the total amount of albumin and globulin present in the blood.[18] These proteins are important liver function markers. Values below the normal threshold usually are associated with nutritional deficiency, liver and kidney disease, or prolonged hemorrhage or anemia. Elevated total protein values can be a marker of chronic inflammation.[19] So, PVR may have adverse effects on the liver because it caused noticeable elevation in the total protein level. Albumin is the major portion of the total protein and one of the most important proteins in human physiology, has the multiple functions including maintaining the plasma volume, transporting hormones-vitamins-drugs and exerting a powerful antioxidant-anti-inflammatory action. Low albumin levels are a warning and an indication that further investigation may be warranted. In addition, albumin is primarily produced by the liver, that is why a low albumin level may indicate liver disease or damage.[20] There was a non-significant reduction in the serum albumin level after chronic use of PVR in male rats which indicates the chance of hepatotoxicity.

Bilirubin is an orange-yellow pigment of bile that is produced from the degradation of various heme containing proteins, especially from hemoglobin catabolism.[21] It is a potentially toxic substance. However, the body has developed some mechanisms for its safe detoxification and deposition.[22] Although bilirubin is a well-established marker of liver function, further analysis and investigation is required for proper diagnosis. A high level of bilirubin may indicate some problems in the liver or gallbladder. Lack of any change in the bilirubin level in the PVR treated male rats reveals functional integrity of hepatocyte canalicular membrane.

ALT or sGPT (Serum glutamic-pyruvic transaminase) or ALT is an enzyme predominantly found in the liver as well as in the kidneys, hearts and muscle cells. This enzyme is used to convert food into energy. However, too little or too much quantity in the body may cause certain health problems. An increase in ALT serum levels indicates definite liver cell injury or liver toxicity.[23] Elevated level of sGPT might be an indication of liver damage. But in this study the PVR treated male rats have shown a very highly significant decrease in the ALT activities. Aspartate aminotransferase (AST) or sGOT (serum glutamic-oxaloacetic transaminase) is an enzyme, which is involved in maintaining several body functions. This enzyme is primarily found in the muscles, liver, and heart. It may become elevated in response to tissue damage or disease, making it a useful indicator for liver health. Elevated level of sGOT might be an indication of liver damage.[24] But in this study, the PVR treated male rats have shown a very highly significant decrease in the AST enzyme activities.

The ratio of the serum aspartate to alanine amino-transferase levels (AST/ALT) is often used as a clue to the etiology of the underlying liver disease.[25]

LDH is an important enzyme of the anaerobic metabolic pathway and found in almost all of the body's cells, but only a small amount of it is usually detectable in the blood. Conditions that can cause increased LDH in the blood may include liver disease, anemia, heart attack. However, to get more information, alternate assays such as CK for muscle, ALT for liver, troponin for heart diseases, etc. are needed.[26] Elevated level of LDH activity might be an indication of cardiac damage. But in this study, the PVR treated male rats have shown a very highly significant decrease in LDH.

ALPs are a group of isoenzymes located on the outer layer of the cell membrane which has a crucial role in the body particularly in liver and bone function. It helps break down proteins and is involved in various cellular processes, including bone mineralization and bile production. This enzyme is mostly found in the liver and bone. Over 80% of the ALP in serum originates from the liver and bone.[27] High ALP usually means that either the liver has been damaged or a condition causing increased bone cell activity is present. Increased ALP level can be associated with certain diseases like hepatitis, blockade of bile ducts, liver cancer, Paget's disease etc.

However, increased level of ALP is mainly associated with liver damage or bone disorder. Therefore, the elevated level of ALK-Pase activity might be an indication of liver damage. But in this study the PVR treated male rats have shown a very highly significant decrease in ALP enzymatic activity.

GGT is an enzyme found in the liver, other organs, and in the blood, that plays a role in transferring the gamma-glutamyl group of glutathione to form glutamate, potentially aiding in protection against oxidative stress induced by factors like ethanol metabolism. Serum GGT has been widely used as an index of liver dysfunction and marker of alcohol intake. Conditions that increase serum GGT, such as liver damage, obstruction in bile duct, high alcohol consumption, and use of enzyme-inducing drugs.[28] Therefore, an elevation in the activity of sGGT might be an indication of liver damage, especially the susceptibility of liver as an organ to alcohol ingestion. And in this study the PVR treated male rats have shown a negligible increase in sGGT activity in comparison to the control male rats revealing though negligible yet slight increase in susceptibility of liver towards alcohol content of this Asava preparation.

## CONCLUSION

In conclusion, the present investigation has shown that total protein increased, and Albumin decreased, whereas Bilirubin remains unchanged in the male rats. In addition to that, a significant drop was observed in most of the remarkable enzymes such as ALT, AST, ALP. These findings suggest that there might be potential therapeutic implications, especially in conditions where reduced enzymatic activities require. However, broader clinical research is essential to fully elucidate the hepatobiliary implications of PVR on chronic administration.

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