

# Antihepatotoxic effect of golden berry (*Physalis peruviana* Linn.) in carbon tetrachloride (CCl<sub>4</sub>) intoxicated rats

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**Abstracts:** Liver is the main site in the body for intense metabolism and excretion. A number of chemicals and drugs which are used routinely cause liver damage. The present study investigates the antihepatotoxic effect of *Physalis peruviana* whole ripe fruit, water and ethanol extracts of fruit in normal as well as in carbon tetrachloride (CCl<sub>4</sub>) intoxicated rats. The CCl<sub>4</sub> treated rats showed marked elevation in liver enzymes: alanine transaminase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase and other biochemical parameters: bilirubin, creatinine and urea, thus indicating liver injury. Whereas animal treated/fed with various preparations of *Physalis peruviana* showed significant lowering effect ( $p < 0.05$ ) in the elevated levels of serum markers like ALAT, ASAT, ALP, LDH, creatinine, urea and bilirubin indicating the protection against hepatic cell damage. The water extract of *Physalis peruviana* showed highest activity in both rat models while ripe fruit and ethanol extract showed moderate activity compared to standard drug.

**Keywords:** *Physalis peruviana*; antihepatotoxic; CCl<sub>4</sub>; transaminases; liver injury.

## INTRODUCTION

Liver is the vital organ of the body performing a number of functions including metabolism, removal of biological toxins and medicinal agents. These functions are associated with the disturbance of hepatocyte biochemistry and generation of ROS (reactive oxygen species) (Fernandez-Checa and Kaplowitz, 2005). A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage (Meyer and Kulkarni, 2001). Most commonly used chemicals are carbon tetrachloride (CCl<sub>4</sub>) and acetaminophen. Carbon tetrachloride (CCl<sub>4</sub>) is environmental pollutant which causes hepatotoxic effects by producing centrilobular necrosis and steatosis. It is reported that 6 hours after the treatment of CCl<sub>4</sub> (single dose, 2ml/kg) caused liver toxicity indicated by disturbance in biochemical marker enzymes in the serum (Vanitha, *et al.*, 2007). The mechanism of hepatic injury by CCl<sub>4</sub> involves lipid peroxidation of membrane bound fatty acids which results in destructing the cell membrane and the intracellular organelles of the hepatocyte (De-Groot and Noll, 1986).

Herbal medicines are in great demand in the developed as well as developing countries for the primary health care because of their wide biological and medicinal activities, higher safety margins and lesser cost (Chattopadhyay and Bhattacharyya, 2007). *Physalis peruviana* is a medicinal plant widely used in folk medicine as anticancer antipyretic, immune modulatory, antispasmodic, diuretic, antiseptic, sedative analgesic, helping to fortify the optic

nerve, throat relief, elimination of intestinal parasites, amoebas as well as albumin from kidneys and also for treating diseases such as malaria, asthma, dermatitis, rheumatism and hepatitis (Wu, *et al.*, 2004ab; Arun and Asha, 2007; Hassanien, 2011). *Physalis peruviana* has also been reported to have hepatoprotective activity (Arun and Asha, 2007; Chang, *et al.*, 2008). The aim of this study was to analyze the protective effect of *Physalis peruviana* fruit extracts (water, ethanol) and whole ripe against liver injury.

## MATERIALS AND METHODS

### Chemicals

All kits for the determination of serum Aspartate transaminase (ASAT), Alanine transaminase (ALAT), and Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH) and urea were purchased from Ecoline, Germany. Creatinine, bilirubin total and direct assay kit were purchased from Merck (private) Limited, France. Carbon tetrachloride (CCl<sub>4</sub>) was purchased from Merck (private) limited, France. Liv-52 syrup (Himmont Laboratories (pvt) Limited, under the license of Himalaya Drug Company, Dubai, U.A.E) was used as a standard drug. All the solvents used in this study were of analytical grade.

### Plant Material

The mature and fresh fruits of *Physalis peruviana* L. (5 kg) were purchased from local market of Karachi, Pakistan during the month of March, 2011. Dr. Rubina Abid, Department of Botany, University of Karachi identified the plant material.

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**Table 1:** Effect of whole ripe fruit, water and ethanol extracts of *Physalis peruviana* fruit on blood serum enzymes (ALAT, ASAT, ALP and LDH) in normal and CCl<sub>4</sub> intoxicated rats.

Groups	Treatments	ALP (IU/L)		ASAT (IU/L)		ALAT (IU/L)		LDH (IU/L)	
		Normal	CCl <sub>4</sub>	Normal	CCl <sub>4</sub>	Normal	CCl <sub>4</sub>	Normal	CCl <sub>4</sub>
1	Control	113±2.6 <sup>c</sup>	251±10.5 <sup>a</sup>	114.7±3.8 <sup>a</sup>	323±4 <sup>a</sup>	23±1.5 <sup>bc</sup>	95±5.5 <sup>a</sup>	251±8.5 <sup>c</sup>	574±30.7 <sup>a</sup>
2	Liv52 (500mg/kg)	108.7±2 <sup>c</sup>	152±9.2 <sup>c</sup>	88±2.6 <sup>d</sup>	201±7 <sup>c</sup>	22±1 <sup>bc</sup>	53±2.3 <sup>d</sup>	337±11.5 <sup>b</sup>	462±8 <sup>c</sup>
3	Ripe fruit (23g/kg)	85.7±2 <sup>d</sup>	162±2.6 <sup>c</sup>	107.3±1.5 <sup>b</sup>	219±4 <sup>b</sup>	31±1.5 <sup>a</sup>	72±1.7 <sup>b</sup>	348.3±7.6 <sup>b</sup>	417±10.7 <sup>d</sup>
4	Water extract (250mg/kg)	132.3±11 <sup>b</sup>	89±1.7 <sup>d</sup>	96.3±2 <sup>c</sup>	185±4.2 <sup>d</sup>	20±1.5 <sup>c</sup>	35±1 <sup>e</sup>	379.6±7.5 <sup>a</sup>	521±13 <sup>b</sup>
5	Ethanol extract (500mg/kg)	215±6 <sup>a</sup>	184±7.2 <sup>b</sup>	114.7±1.5 <sup>a</sup>	228±11 <sup>b</sup>	24±1 <sup>b</sup>	62±2.5 <sup>c</sup>	369±5.6 <sup>a</sup>	526±14.8 <sup>b</sup>

Data represents the means ± SD

Values presented in one column with same superscript were not significantly differing from each other by Duncan's multiple range test.

**Table 2:** Effect of whole ripe fruits, water and ethanol extracts of *Physalis peruviana* fruit on different biochemical parameters (creatinine, urea, bilirubin total & direct) in normal and CCl<sub>4</sub> intoxicated rats.

Groups	Treatments	Creatinine (mg/dl)		Urea (mg/dl)		Bilirubin total (mg/dl)		Bilirubin direct (mg/dl)		Bilirubin indirect (mg/dl)	
		Normal	CCl <sub>4</sub>	Normal	CCl <sub>4</sub>	Normal	CCl <sub>4</sub>	Normal	CCl <sub>4</sub>	Normal	CCl <sub>4</sub>
1	Control	0.76±0.06 <sup>b</sup>	1±0.06 <sup>a</sup>	34±2 <sup>a</sup>	46.7±1.5 <sup>a</sup>	0.7±0 <sup>a</sup>	1.03±0.06 <sup>a</sup>	0.2±0 <sup>bc</sup>	0.33±0.057 <sup>a</sup>	0.5±0 <sup>a</sup>	0.7±0 <sup>a</sup>
2	Liv-52 (500mg/kg)	0.93±0.15 <sup>a</sup>	0.83±0.052 <sup>c</sup>	35±1.1 <sup>a</sup>	44±2.3 <sup>a</sup>	0.7±0.057 <sup>a</sup>	0.77±0.05 <sup>b</sup>	0.3±0 <sup>a</sup>	0.23±0.057 <sup>ab</sup>	0.44±0.05 <sup>ab</sup>	0.53±0.06 <sup>b</sup>
3	Whole ripe fruit (23g/kg)	0.73±0.15 <sup>b</sup>	0.93±0.055 <sup>b</sup>	25±1 <sup>b</sup>	38.7±2 <sup>b</sup>	0.66±0.05 <sup>a</sup>	0.76±0.057 <sup>b</sup>	0.23±0.05 <sup>abc</sup>	0.23±0.057 <sup>ab</sup>	0.44±0.05 <sup>ab</sup>	0.53±0.06 <sup>b</sup>
4	Water extract (250mg/kg)	0.8±0 <sup>ab</sup>	0.86±0.057 <sup>bc</sup>	24±1.5 <sup>b</sup>	31.7±1.5 <sup>c</sup>	0.57±0.05 <sup>b</sup>	0.66±0.06 <sup>b</sup>	0.16±0.06 <sup>c</sup>	0.16±0.057 <sup>b</sup>	0.4±0 <sup>b</sup>	0.5±0 <sup>b</sup>
5	Ethanol extract (500mg/kg)	0.76±0.06 <sup>b</sup>	0.8±0 <sup>c</sup>	36±2.5 <sup>a</sup>	41±1 <sup>b</sup>	0.67±0.051 <sup>a</sup>	0.73±0.057 <sup>b</sup>	0.26±0.054 <sup>ab</sup>	0.23±0.057 <sup>ab</sup>	0.4±0 <sup>b</sup>	0.5±0 <sup>b</sup>

Data represents the means ± SD

Values presented in one column with same superscript were not significantly differing from each other by Duncan's multiple range test.

### Preparation of Fruit Extracts

#### Ethanol Extract

Ethanol extract was prepared by crushing *Physalis peruviana* (fruit) by mortar and pestle, the crushed material was soaked in distilled ethanol for 1 week at room temperature. Ethanol extract was filtered by cotton wool. Extract was dried under reduced pressure on rotary vacuum evaporator (Buchi R-200, Japan) at 40°C in order to get gummy paste. The residues were re-extracted 3 times under the same conditions.

#### Water Extract

One hundred gram *Physalis peruviana* (Fruit) was first crushed by mortar and pestle and homogenized. The extract was filtered through Whatman No.1 filter paper and the residues were re-extracted until the colorless filtrate was appeared. The filtrate was stored in refrigerator and freeze dried on Eyela FD-I freeze drier, powder form was hygroscopic in nature so immediately stored in air tight sample vial at -20°C until used.

### Experimental Design

Healthy Wistar female rats (150-160g) were purchased from Dow University of Health Sciences, Karachi, Pakistan. They were housed (4 rats per cage) in an environmentally controlled temperature (22±2°C) with a 12:12-hr light/dark cycle. Animals were fed with a standard laboratory diet and had free access to water. After acclimatization period, rats were divided into 5 different groups, each group was comprising of 8 rats. Rats in group 1 were served as control rats (orally given distilled water, 2ml/kg b.w. daily for 6 days), group 2 rats were given standard drug Liv-52 at a dose of 500 mg/kg b.w. (since dose of 250 mg/kg b.w. was not effective). Animals of group 3 were allowed to feed whole ripe fruit of *Physalis peruviana*, 23g/kg b.w. Group 4 animals were orally administered with water extract of *Physalis peruviana* (250 mg/kg b.w.) and group 5 rats were orally administered with ethanol extract of *Physalis peruviana* at a dose of 250 mg/kg b.w. but dose of 250 mg/kg b.w. of ethanol extract was not effective, so the amount of dose

was increased to 500 mg/kg b.w. All the treatments were given to rats for 6 days. On the last day, 24 hours before decapitation, half rats from all groups were separated and injected (i.p.) with CCl<sub>4</sub> (1:1 in olive oil, 2ml/kg) while other half were injected (i.p.) with olive oil at a dose of 2ml/kg. All the animals were fasted overnight before decapitation. The experiment was repeated twice.

### Biochemical Analysis

Blood was collected in the sterilized centrifuge tubes and allowed to clot at room temperature. Serum was separated after centrifugation at 3000 rpm for 10 minutes and stored at -20°C till analysis. Serum samples collected from different groups were analyzed for Aspartate transaminase (ASAT), Alanine transaminase (ALAT) and Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), urea, creatinine, bilirubin (Bil-total & Bil-direct). All the samples were analyzed on Microlab 300 (semi-automated analyzer), Merck (private) limited.

### STATISTICAL ANALYSIS

Statistical analysis was performed using one way analysis of variants (ANOVA). Differences among the mean values were found by Duncan's multiple range tests. The results were expressed as mean  $\pm$  SD. *P*-value less than 0.05 ( $p < 0.05$ ) was considered significant.

### RESULTS

The serum level of hepatic enzymes (ALAT, ASAT, ALP, LDH) and other biochemical parameters (bilirubin, creatinine and urea) were elevated after the administration of CCl<sub>4</sub> in hepatotoxic treated rats as compared to normal control rats. The hepatoprotective activity was found in rats treated with *Physalis peruviana* whole ripe fruit (23g/kg b.w.), fruit extracts (water 250mg/kg and ethanol 500mg/kg b.w) against CCl<sub>4</sub>-induced liver injury. The hepatoprotective activity was compared with the standard drug Liv-52. Since Liv-52 at the dose level of 250 mg/kg did not lower elevated level of hepatic enzymes and other biochemical parameters in CCl<sub>4</sub> intoxicated rats. Therefore 500mg/kg b.w. of standard drug was used. Water extract of *Physalis peruviana* significantly decreased ( $p < 0.05$ ) serum enzymes level, bilirubin, creatinine and urea as compared to standard drug in both normal and CCl<sub>4</sub> intoxicated rat models whereas whole ripe fruit and ethanol extract showed moderate activity as compared to standard drug (table1 and 2).

### DISCUSSION

Liver is vital organ of body, when the liver is damaged due to any toxic chemical, infectious agent or drug overdose, a number of blood biochemical indicators increased, such as ALAT, ASAT, ALP, LDH, bilirubin, and others. ALAT being more sensitive and highly specific, it is commonly used for liver function

evaluation, especially for acute liver damage. These markers are cytoplasmic in origin and released into the circulation after cellular damage (Lin *et al.*, 2000; Mohan, 2002).

High levels of ASAT indicate liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury (Drotman and Lawhorn, 1978). We found significant increase in the levels of serum transaminases (ASAT and ALAT) after CCl<sub>4</sub> intoxication. These enzymes levels were reduced in rats treated with water extract alone as compared to normal control rats. Administration of *Physalis peruviana* ripe fruit and extracts restored the elevated levels of ASAT, ALAT enzymes after CCl<sub>4</sub> administration. Increase in serum level of ALP is due to increased synthesis, in the presence of increasing biliary pressure. After the administration of CCl<sub>4</sub>, ALP is significantly ( $p < 0.05$ ) increased in serum. The significant ( $p < 0.05$ ) restoration of this enzyme level was found in rats treated with ethanol and water extracts and ripe fruit, but highest hepatoprotective activity was found in water extract as compared to standard drug.

The elevation in the level of creatinine and bilirubin has been reported in carbon tetrachloride, acetaminophen induced hepatotoxicity (Yamaguchi *et al.*, 1996; Ellenhorn, 1997). Bilirubin is conventional indicator of liver diseases and these biochemical restorations may be due to the promotion of its glucuronidation (Recknagel *et al.*, 1989). Both the extracts and ripe fruit treated rats after the administration of CCl<sub>4</sub> showed significant ( $p < 0.05$ ) decrease in bilirubin levels compared with CCl<sub>4</sub> control rats. The elevated level of creatinine and urea in serum shows the liver or kidney damage. Present study showed the increased levels of creatinine and urea in the serum of CCl<sub>4</sub> intoxicated rats as compared to control rats. The study recorded significant ( $p < 0.05$ ) decrease in the levels of creatinine and urea in all treated groups of *Physalis peruviana* and Liv-52 pre-treated groups. Water extract showed highest protection as compared to standard drug followed by other *Physalis peruviana* treated groups. A possible mechanism of the *Physalis peruviana* whole ripe fruit, water and ethanol extracts as hepatoprotective may be due to its antioxidant effect or inhibition of cytochrome P450 which impair the bioactivation of CCl<sub>4</sub> (Recknagel, 1989) into their corresponding reactive species. It is reported that different compounds: 28-hydroxywithanolide, withanolides, phygrine, kaempferol, and quercetin di- and tri-glycosides are present in *Physalis peruviana* (Elliger, *et al.*, 1992; Keith, *et al.*, 1992; Dinan, *et al.*, 1997). These compounds have a strong antioxidant property and prevent oxidative damage to liver microsomes and hepatocytes (Wang, *et al.*, 1999). It has been reported that crude water extract of *Physalis peruviana* contains phytochemicals, flavonoids, saponins and phenols (Arun and Asha, 2007). Flavonoids are powerful antioxidants against free radicals and are described as free-radical scavengers (Pal, *et al.*, 2009).

The antioxidant property is claimed to be one of the mechanisms of hepatoprotective impact (Bhatt and Bhatt, 1996).

## CONCLUSION

In this era of science and technology the demand for therapeutic drugs from natural products is increasing day by day, due to their effective therapeutic action and lack of side effects. The effect of *Physalis peruviana* against hepatotoxin mediated liver injury was studied. In all groups, water extract of the fruit showed most significant activity. Since golden berry has hepatoprotective effect it is suggested that it may be used against liver diseases.

## ABBREVIATIONS

L. Linn, CCl<sub>4</sub>. Carbon tetrachloride, b.w. Body weight, ROS. Reactive oxygen species, ml. Milliliter, Kg. Kilogram, i.p. Intraperitoneal, ALAT. Alanine transaminase, ASAT. Aspartate transaminase, ALP. Alkaline phosphatase, LDH. Lactate, dehydrogenase, U.A.E United Arab Emirates, Rpm. Rotations per minute, SD. Standard deviation

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