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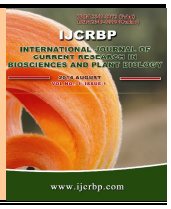

Original Research Article

Hepatoprotective Activity of *Physalis minima* against Paracetomal Induced Hepatic Damage in Rats

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Abstract

The present study is determination of the hepatoprotective activity of *Physalis minima* against paracetamol-induced hepatic injury in rats. Ethanolic extracts of *P. minima* were investigated for phytochemical, antioxidant, hepatoprotective activity and antibacterial activity. The various phytochemical tests were performed to know the active components present in the *P. minima* such as flavonoids, glycosides, saponins, tannins, amino acids and Terpenoids. The antibacterial activity of *P. minima* against *Escherichia coli, Staphylococcus aurous, Bacillus subtilis,* and *Klebsiella pneumoniae* were evaluated using agar well diffusion technique. The diameter zone of inhibition was taken as an indicator of antibacterial effect. Hepatoprotective activity of paracetomal treated rats showed significant increase in total bilirubin (0.8mg/dl), SGPT (320U/L), SGOT (212U/L) as compared to control and other experiments. The results clearly indicated that the leaves of *P. minima* were possessing hepatoprotective activity in comparison with standard hepatoprotective drug silymarin.

Keywords

Antibacterial activity

Hepato-protective

Kelbsiella pneumoniae

Paracetamol

Physalis minima

Phytochemicals

Introduction

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Manokaran et al., 2008). Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Vijay et al., 2009).

Liver injury and altered liver functions are directly proportional to doses of drugs or chemicals. It sis documented that both paracetamol (PCM) and carbon tetrachloride (CCl_4) elicit centritubular necrosis largely due to reactive toxic metabolites formation (Shrinivas and Suresh, 2012). In the absence of reliable liver- protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief.

The traditional system of medicine like Ayurveda and Siddha system of medicine, Unani system, Chinese system of medicine, Kampoo (Japanese) system of medicine have a major role in the treatment of liver ailments (Vijay et al., 2009). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Salma et al., 2009).

In recent years, many researchers have studied the effect of herbal medicine used traditionally to improve liver function and also treat hepatic disorders. Treating liver diseases with plant derived compounds seems highly attractive. Some potent herbs with established hepatoprotective activity are: *Silybum marianum, Glycyrrhiza glabra, Phyllanthus niruri, Andrographis paniculata* and *Tinospora coradifolia* (Shrinivas and Suresh, 2012).

Physalis minima of Solanaceae family is an annual herb having 0.5-1.5 m height with a very delicate purple- tinged stem and leaves. It is found throughout India, Baluchistan, Afghanistan, Tropical Africa, Singapore, Malaysia and Australia and is reported as one of the important medicinal plants in Indian traditional system of medicines. The common name of *Physalis minima* is native gooseberry, country gooseberry, wild Cape gooseberry (Daya and Vaghasiya, 2012).

Physalis minima plant parts are used leaf, stem, root, and fruit. The chemical composition of the fruit is good amount of vitamin C. The fruits contain sugars, reducing sugars, non- reducing sugars, tannins and pectin. In additions, it contain protein, minerals, etc.The plant contains withasteroids, physalindicanols, withaminimin and withaphysalin, -O-glucosides of kaempferol and quercetin, in addition to beta-sitosterol and its glucoside. Phytochemical constituents like alkaloids, anthraquinones, flavonoids, cardiac glycosides, phenols, quinones, reducing sugars, saponins, steroids, starch, tannin and terpenoids of the stem, leaf and unripe fruit (Nathiya and Dorcus, 2012).

Liver, an important organ actively involved in many metabolic functions, is the frequent target for number of toxicants. The use of herbal medicine in the treatment of diseases is increasing worldwide (Samudram et al., 2008).

All three pathways yield final products that are inactive, non-toxic, and eventually excreted by the kidneys. In the third pathway, however, the intermediate product NAPQI is toxic. NAPQI is primarily responsible for the toxic effects of paracetamol; this constitutes an example of toxication (Hendrickson and Bizovi, 2006).

As the principal para-aminophenol derivative in use, paracetamol replaced its predecessors in the same therapeutic class, acetanilide and phenacetin, as the analgesic-antipyretic of choice for patients in whom salicylates and other non steroidal anti-inflammatory drugs are contraindicated. Due to its weak inhibition of cyclo-oxygenase, paracetamol is not indicated as an anti-inflammatory drug (Forte, 2002).

Paracetamol toxicity is caused by excessive use or overdose of the analgesic drug paracetamol (called acetaminophen in North America). Mainly causing liver injury, paracetamol toxicity is one of the most common causes of poisoning worldwide. In the United States and the United Kingdom it is the most common cause of acute liver failure (Larson et al., 2005).

Silymarin is an antioxidant flavonoid $C_{25}H_{22}O_{10}$ consisting of a mixture of three isomers isolated from seeds of the milk thistle, held to have properties protecting the liver from or clearing it of toxins, and used in dietary supplements and herbal remedies. Scientific Name: Milk Thistle. Other Names: Cardui mariae, *Carduus marianum*, Holy Thistle, Lady's Thistle, Legalon, Marian Thistle, Mariendistel, Mary Thistle, Our Lady's Thistle, Silimarina, Silybin, *Silybum marianum*.

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are

synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and in the human therapy, veterinary, agriculture, scientific research and countless other areas. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Yadav and Agarwal, 2011).

Antioxidants are radical scavengers, which product the human body against free radicals. Free radical also induces liver damage. Likewise, metabolism of certain drugs like paracetamol (PCM), produce free radicals, which cause liver damage. Several flavonoids have been reported to possess antioxidant and hepatoprotective properties (Bhandansh et al., 2010).

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases. Many coumarin derivatives have special ability to scavenge reactive oxygen species (ROS)—free radicals, such as hydroxyl radicals, superoxide radicals or hypochlorousacid, and to influence processes involving free radical-injury (Rajesh Patel and Patel Natvar, 2011). Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and Cancer. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Khalaf et al., 2008).

Mammalian cells possess elaborate defence mechanisms for radical detoxification. Key metabolic steps are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which destroy toxicperoxides. In addition to antioxidant enzymes, non enzymatic molecules, including thioredoxin, thiols, and disulfide-bonding play important roles in antioxidant defence systems. Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plant extracts. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, b-carotene, and a-tocopherol are known to possess antioxidant potential (Farrukh et al., 2006).

SGOT found principally in the cytoplasm of the liver cells serves as a measure of the integrity of the hepatocytes, and correlates to the number of hepatocytes exaggerated. The leakage of the enzyme into the blood stream indicates the injury to the hepatocytes4.SGPT virtually found in almost every tissue of the body is useful for the production of antioxidants in conditions of oxidative stress (Rani Veena et al., 2011). The present study was hepatoprotective activity of *Physalis minima* against paracetamol induced hepatotoxic rats.

Materials and methods

Collection of Plant Material

The plant material of *Physalis minima* (Solanaceae) were collected from Enathur, Kanchipuram district, TamilNadu, India.

Ethanolic Extract of *Physalis minima*

The leaves were shade dried for 2 weeks and milled into coarse powder by a mechanical grinder. Then the powdered sample was warped in Whatman No-1 filter paper by using thread. Then the powered sample was placed in Soxhlet apparatus and to that 200ml of ethanol. After 6 hours the ethanolic extract was collected and it was used for further studies (Ramalingam et al., 2010).

Phytochemical Screening of *Physalis minima*

Preliminary phytochemical screening of *Physalis minima* was carried out using standard procedures (Bharathi et al., 2008).

Organisms used for Anti- bacterial activity

Bacteria: *Escherichia coli, Staphylococcus aureus, Klepsiella pneumoniae* and *Bacillus subtilis.*

Assessment of Antibactrial activity of *Physalis minima*

The ethanolic leaf extract of *Physalis minima* were tested by the agar well diffusion method. Extracts were dissolved in 10% DMSO (Dimethyl sulfoxide) to a final concentration of 100mg/ml. Petri plates containing 20ml of Muller Hinton Agar medium were seeded with 24h cultures of *E.coli, Klepsiella species, Bacillus subtilis, and Staphylococcus aureus*. Wells were cut into agar and different concentration (50µl and 75µl) of plant extracts were tested and incubation was performed at 37 ^{0} c for 24 hour. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well (Jigna and Sumitra, 2007).

Quantitative analysis of flavonoid

Total flavonoid content in *Physalis minima* samples was determined according to Blasa et al. (2006).

Antioxidant activity of *Physalis minima*

The antioxidant activity was determined by Diphenyl Picryl Hydrazyl (DPPH) radical scavenging, enzymatic antioxidant analysis of superoxide dismutase (SOD) and Catalase (CAT) activities.

DPPH Radical scavenging activity

The DPPH radical scavenging activity was measured by spectrophotometric method. 1ml of ethanolic solution of extract of various concentrations (100 to 500µl) was mixed with 1ml of ethanolic solution of DPPH (200µM). Similarly 1ml of ethanolic solutions of ascorbic acid (200µg/ml) was mixed with 1ml of DPPH solution. A mixture of 1ml of ethanol and 1ml of ethanolic solution of DPPH (200µM) served as control. After mixing, all the solution was incubated in dark for 20 minutes and absorbance was measured at 517nm. The experiments were performed in triplicate and percent scavenging activity was calculated as follows:

Scavenging % = (Absorbance of control-absorbance of test/Absorbance of control) × 100.

Hepatoprotective Activity
Experimental Design

Group-I

Control rats orally received or treated with 0.5% of CMC (Carboxy Methyl Cellulose) (1ml/kg) for seven days.

Group-II

Rats orally received paracetamol (3gm/kg) dissolved in 0.5% CMC (Carboxy Methyl Cellulose) for seven days.

Group-III

Rats are orally received paracetamol followed by administration of silymarin (25mg/kg) dissolved in 0.5% of CMC (Carboxy Methyl Cellulose) for 7days.

Group-IV

Rats orally received paracetamol (3gm\kg body weight) followed by oral administration of *Physalis minima* leaf extract (200mg/kg).

Biochemical estimation

On the 7th day of the start of respective treatment the rats were anaesthetized by light ether anaesthesia and the blood was withdrawn by direct cardiac puncture to the rats. It was allowed to coagulate for 30 minutes and serum was separated by centrifugation at 2500 rpm the serum was used to estimate SGOT, SGPT and Bilirubin.

Results and discussion

The plant material of *Physalis minima* was collected from Enathur, Kanchipuram district, TamilNadu, India is shown in Fig. 1.

Ethanolic Extraction of *Physalis minima*

The ethanolic extract of *Physalis minima* (leaf) were obtained for analysing the various activities of the plants using Soxhlet apparatus.

Phytochemical screening of *Physalis minima*

The various phytochemical tests were performed to know the active components present in the *Physalis minima* such as Flavonoids, Glycosides, Saponin, Tannins, amino acids and Terpenoids (Table 1).

Antibacterial activity of *Physalis minima*

The ethanolic extract of *Physalis minima* (50 µl and 75µl) was added to the wells of Muller-Hinton agar plates inoculated with bacterial cells. The plates were incubated overnight at 37 ^{0} C. The zone of inhibition was measured and calculated from the diameter of the zones obtained the *Bacillus subtilis* was shown the highest inhibitory activity when compared to the other species as shown in Table 2 and Fig. 2.

Quantitative analysis of flavonoids

Quantitative analysis of flavonoids is measured and it was performed triplicates in studies. Total flavonoids content also measured by colorimetric method at 420nm and expressed as quercetin equivalent.

Antioxidant Activity of *Physalis minima*

The antioxidant activity was determined by Diphenyl picryl hydrazyl (DPPH) radical scavenging, enzymatic antioxidant analysis of superoxide dismutase (SOD), Catalase (CAT). The ethanolic extract of *Physalis minima* leaves in graded concentrations was tested for antioxidant activity. It was observed that the test compounds scavenged free radicals in a concentration dependent manner. Maximum percentage inhibition of DPPH radicals by the extract of was about 30% at 500µl concentration. The activity of superoxide dismutase present in the *Physalis minima* sample was 1.66% of pyrogallol auto-oxidation inhibition per minute. Then the activity of catalase present in the *Physalis minima* sample was 0.884% of hydrogen peroxide utilized per minute.

Hepatoprotective Activity of *Physalis minima*

Present investigation of used to evaluating the role of *Physalis minima* on paracetamol induced hepatic damage. Several enzymes are located in the cytosol and released into the blood when the plasma membrane of liver cell damage. There level is estimated with useful marker of hepatocellular damage identification. Hepatic injury is identified by cellular enzymes, such as SGOT, SGPT, and Serum bilirubin.

Fig. 1: *Physalis minima*

A marketed drug silymarin is one of the standard hepatoprotective herbal formulation is collectively called as silibin extracted from *Silybum marianum.*

In the study of hepatoprotective activity of *Physalis minima,* after treatment with paracetamol, a significant increase in levels of SGOT, SGPT and Serum Bilirubin as compared to the control was observed as shown as Table 3 and Fig. 3.

Biochemical Estimation

Group I Control: The basal levels of liver enzymes (SGPT, SGOT) in control were 22U/L and 172U/L respectively. Total Bilirubin levels were o.4mg/dl respectively.

Group II Paracetomal: There was significant increase in Total Bilirubin 0.8mg/dl, and also significant increase in SGPT (320U/L), SGOT (212U/L) as compared to the control.

Group III *Physalis minima*: There was significant decrease in total Bilirubin (0.6mg/dl), and also significant decrease in SGPT (110 U/L), SGOT (104U/L) as compared to the toxic control.

Group IV Silymarin: There was significant decrease in total Bilirubin (0.5mg/dl), and also significant decrease in SGPT (79U/L), SGOT (82U/L), as compared to the toxic control.

Changes in the activity of liver functional marker SGPT and SGOT

Table 3 represents change in the activities of SGPT and SGOT in serum. In this table paracetamol induced liver damage was characterized by increasing SGPT and SGOT levels and return to normal after seven days of administration of *Physalis minima* leaf extracts.

Group II increases the SGPT and SGOT levels when compared to Group IV. After administration of Group III (*Physalis minima* leaf extracts) decreases SGPT and SGOT levels and suggests that it often protects by preserving integrity of hepato cellular membrane against paracetamol induced liver damage.

The paracetamol induced liver damage can be nullified due to the antioxidant effect of *Physalis minima* leaf extract. *Physalis minima* possess free radical scavenging activity. This action of *Physalis minima* can protect the cellular membrane and prevent the leakage of enzymes.

Changes in the activity of serum Bilirubin

Table 3 represents change in the levels of Total bilirubin in serum. In this table paracetamol induced liver damage was characterized by increasing Serum bilirubin levels and return to normal after seven day of administration of *Physalis minima* leaf extracts.

Group II increases the Serum bilirubin levels when compared to Group IV. After administration of Group III (*Physalis minima* leaf extracts) decreases Serum bilirubin levels. This action of *Physalis minima* can protect the cellular membrane from damage their leakage of hepatic enzymes.

Table 1. Phytochemical analysis of *Physalis minima.*

Test carried out	Ethanolic extract
Test for Tannin	–
Test for Saponin	+
Test for Flavonoids	+
Test for Anthocyanin and Betacyanin	+
Test for Quinones	+
Test for Glycosides	–
Test for Cardiac glycosides	+
Test for Acid	+
Test for Steroid	+
Test for Coumarin	+
Test for Phenol	+
Test for Terpenoid	+
Test for Triterpenoid	+

Table 2. Antibacterial activity of *Physalis minima.*

Microorganisms	Zone of Inhibition (mm)		
	Test (50µl)	Test (75µl)	Control
Escherichia coli	13	16	_
Staphylococcus aureus	15	17	_
Bacillus subtilis	17	19	_
Kelbsiella pneumoniae	12	15	_

Table 3. Biochemical parameters in experimental animals.

Experimental Animals	SGPT (U/L)	SGOT (U/L)	Total Bilirubin (mg/ml)
Control (Group I)	22U/L	172U/L	0.4mg/ml
Paracetamol (Group II)	320U/L	212U/L	0.8mg/ml
Physalis minima (Group III)	79U/L	82U/L	0.5mg/ml
Silymarin (Group IV)	110U/L	104U/L	0.6mg/ml

Fig. 2: Antibacterial activity of *Physalis minima* (A: *Klebsiella,* B: *Staphylococcus,* C: *E. coli,* D: *Bacillus subtilis*).

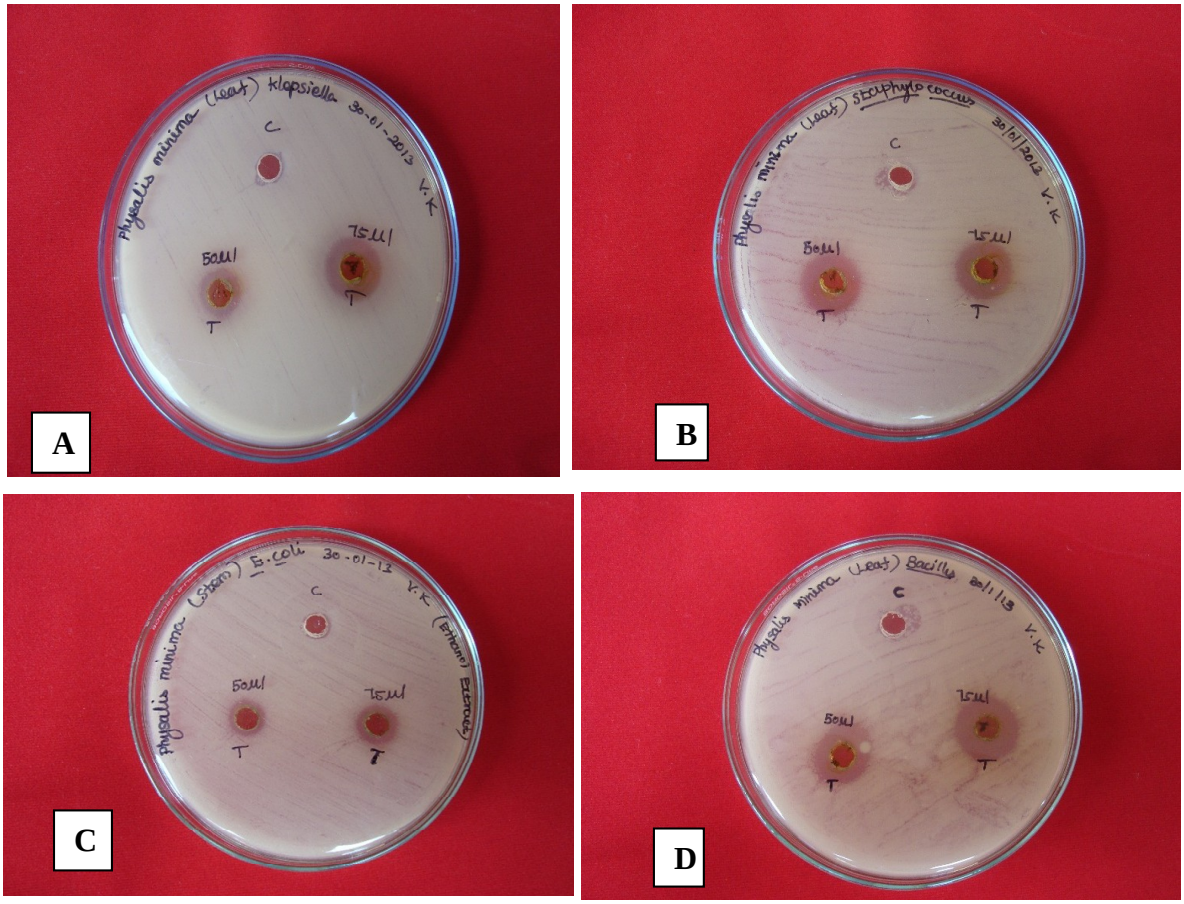
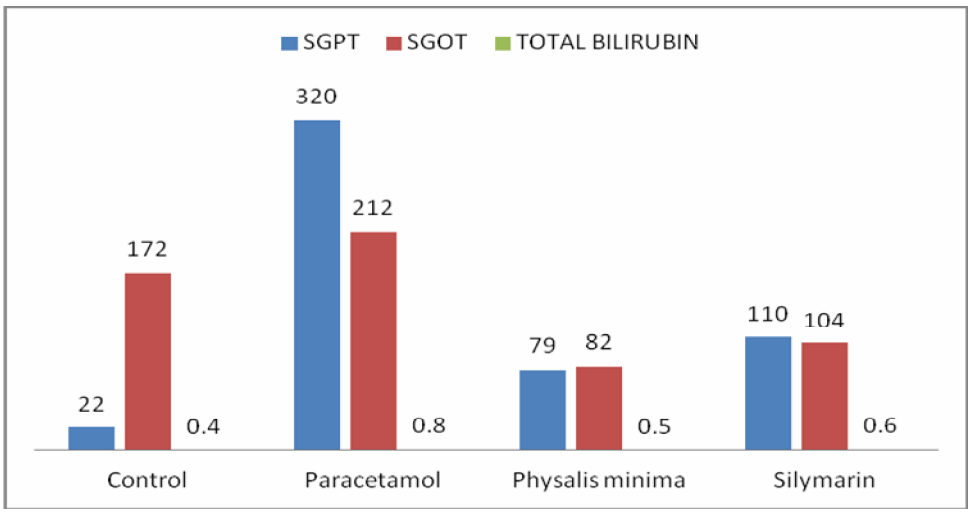


Fig. 3: Biochemical parameters observed in experimental rats.



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