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# Hepatoprotective Effect of the Total Alkaloid Fraction of *Solanum pseudocapsicum* Leaves

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## Abstract

The total alkaloid fraction of the methanol extract of leaves of *Solanum pseudocapsicum* was tested for its hepatoprotective activity against $CCl_4$ induced toxicity in freshly isolated rat hepatocytes, HepG2 cells and animal models. The total alkaloid fraction was able to normalise the levels of aspartate amino transferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), triglycerides (TGL), total proteins, albumin, total bilirubin and direct bilirubin, which were altered due to $CCl_4$ intoxication in freshly isolated rat hepatocytes and also in animal models. The anti-hepatotoxic effect of the total alkaloid fraction was observed at very low concentrations (6–10 µg/ml) and was found to be superior to that of the standard used. A dose dependent increase in the percentage viability was observed when $CCl_4$ exposed HepG2 cells were treated with different concentrations of the total alkaloid fraction. The highest percentage viability of HepG2 was observed at a concentration of 10 µg/ml. Its *in vivo* hepatoprotective effect at 20 mg/kg body weight was comparable with that of the standard at 250 mg/kg body weight. The total alkaloid fraction merits further investigation to identify the active principles responsible for the hepatoprotective properties. The results from the present investigation also indicate well correlation between the *in vivo* and *in vitro* studies.

**Keywords:** *Solanum pseudocapsicum*, hepatoprotective, total alkaloids, hepatocytes, HepG2.

## Introduction

*Solanum pseudocapsicum* Linn. (Solanaceae) is an erect, highly branched, non-spiny, 0.6–1.2 m high, bushy shrub (Anonymous, 1989). In homeopathy, it is being used to treat acute pains in the lower abdomen (Boericke, 1927). It is known for its anti-bacterial (Mitscher et al., 1976), hypertensive, anti-spasmodic (Dhar et al., 1973) and anti-viral (Van den Berghe et al., 1978) activities. Solanocapsine, solanocasine, solacapine and other steroidal alkaloids have been isolated from this plant (Chakravarty et al., 1984). Several plants belonging to the genus *Solanum* are known to possess liver protective effects (Lin et al., 1995; Sultana et al., 1995; Grace & Salen, 1996; Gan et al., 1993; Chun et al., 1987; Chun & Kim, 1989). However, the hepatoprotective effect of the plant *Solanum pseudocapsicum* has not been determined. Hence, the present study was intended to investigate the *in vitro* and *in vivo* anti-hepatotoxic effects of the total alkaloid fraction of the leaves of *Solanum pseudocapsicum*.

## Materials and methods

### Materials

All chemicals were obtained from SD Fine Chemicals, Mumbai. 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), collagenase, insulin, dexamethasone, minimum essential medium (MEM), Ham's F12 medium and antibiotics were purchased from Sigma Chemical Co., St. Loius, MO, USA. Ecoline diagnostic kits were purchased from E – Merck, India. LIV – $52^{(R)}$, a marketed formulation, was used as a standard. The human liver derived HepG2 cell line was obtained from National Centre for Cell Science, Pune, India.

### Plant material

Mature leaves of *Solanum pseudocapsicum* were collected from the fields in and around Government Arts College,

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Ootacamund in the month of May 2000. The plant was authenticated by comparing it with authentic specimen preserved at Survey of Medicinal Plants and Collection Unit, Government Arts College, Ootacamund (Voucher No: 7345).

### Preparation of the plant extract and isolation of the total alkaloid

The fresh mature leaves (240 g) were subjected to a single extraction in a Soxhlet extractor using methanol (1 L) for 18–20 h. The extract was then concentrated to dryness under reduced pressure and controlled temperature to yield a dark green semisolid mass (14.8 g, 6.16%), which was preserved in refrigerated conditions. The total alkaloid fraction (yield 460 mg, 0.19%) was isolated from this extract using a conventional procedure (Trease & Evans, 1978).

### Preparation of suspensions

The total alkaloid fraction of *Solanum pseudocapsicum* was dissolved in DMSO and the volume was made up to 10 ml with Ham's F-12/MEM to obtain a stock solution of 1 mg/ml concentration and stored at –20 °C prior to use. Further dilutions were made to obtain different concentrations ranging from 6–10 µg/ml with respective media and used for i*n vitro* investigations. A suspension of the standard powder was also prepared (250 µg/ml) in a similar manner. The total alkaloid fraction and the standard powder were suspended in sodium CMC (0.3%) in distilled water separately and used for *in vivo* investigations.

## Hepatoprotective effect of the plant extract in freshly isolated rat hepatocytes

### Isolation and culture of hepatocytes

Liver cells were isolated by a modified procedure of Seglen (1994). The calcium-free HEPES buffer and collagenase solutions were warmed in a water bath (37 °C). The abdomen of the rat was opened under phenobarbital sodium (35 mg/kg body weight) anesthesia. A midline incision was made and a loosely tied ligature was placed around the portal vein approximately 5 mm from the liver and the cannula was inserted up to the liver and then the ligature was tightened and heparin was injected into the femoral vein (1000 IU). The inferior venacava was cut below the renal vein. Perfusion was performed for 20 min (37 °C) with calcium free HEPES buffer, which contained 1% bovine serum albumin fraction V at a flow rate of 30 ml/min. The liver swells during this time, slowly changing its color from dark red to grayish white. The swollen liver was then perfused with TPVG solution (50 ml) followed by perfusion with calcium free HEPES buffer, which contained additional collagenase solution (0.075%) and calcium chloride (4 mM) at a flow rate of 15 ml/min for 20 min.

After the perfusion, the lobes were removed and transferred into a sterile petri dish containing calcium-free HEPES buffer and dispersed gently. It was transferred into a sterile conical flask and the crude cell suspension was stirred with the help of a magnetic stirrer for 5 min to release hepatocytes into the solution. The cell suspension was filtered through a nylon mesh (250 µ) and the preparation was centrifuged at 1000 rpm for 15 min. The supernatant was aspirated off and the loosely packed pellet of cells was gently re-suspended in calcium free HEPES buffer. This washing procedure was repeated three times. Cell viability was determined by the Trypan blue dye exclusion method (Freshney, 2000). These isolated hepatocytes were cultured in Ham's F12 medium, supplemented with 10% newborn calf serum, antibiotics, $10^{-6}$ M dexamethasone and $10^{-8}$ bovine insulin. The cell suspension was incubated at 37 °C for 30 min in a humidified incubator under 5% $CO_2$.

### Carbon tetrachloride induced *in vitro* hepatocytes injury

Carbon tetrachloride induced hepatocytes injury assay was carried out. After an incubation of 24 h, the hepatocytes were exposed to the fresh medium containing $CCl_4$ (1%) along with/without various concentrations of the total alkaloid fraction or the medium alone (as normal). After 60 min of $CCl_4$ challenge, concentrations of aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase (ALP), triglycerides (TGL), total proteins, albumin, total bilirubin and direct bilirubin in the medium were measured as an indication of hepatocytes necrosis using Ecoline diagnostic kits (Yoshinobu et al., 1983).

### Hepatoprotective effect in HepG2 cell line

The screening of hepatoprotective activity was based on the protection of human liver derived HepG2 cells against $CCl_4$ induced damage (Ira et al., 1997) determined by estimating mitochondrial synthesis using tetrazolium assay (Ke et al., 1999). HepG2 cells were routinely grown and subcultured as monolayers in DMEM supplemented with 10% newborn calf serum. The experiments in this investigation were conducted with cells that had been initially batch cultured for 10 days. At this stage, the cells were harvested and plated at approximately 30,000 cells/well in 96 well microtitre plates (Nunclon) and left to rest for 24 h at 37 °C in a humidified atmosphere of 5% $CO_2$. The cells were then exposed to toxicant (medium containing 1% $CCl_4$) along with/without various concentrations of the total alkaloid fraction or the medium alone (as normal) (Ira et al., 1997). At the end of the period, cytotoxicity was assessed by estimating the viability of HepG2 cells by MTT reduction assay (Ke et al., 1999). After a 1 h incubation, the test solution from each well was removed by aspiration and replaced with 50 µl of MTT prepared in MEM without phenol red (MEM-PR). The plates were gently shaken and incubated for 3 h at 37 °C in a humid-

ified 5% $CO_2$ atmosphere. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at 540 nm.

### *In vivo* hepatoprotective effect

Colony bred Wistar strain adult albino rats (180–200 g) of either sex were used for the investigations. All the animals were maintained under standard husbandry conditions with food and water *ad libitum*. The experimental procedures were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai (Proposal No. 19/29, JSSCP, Ootacamund). The animals were divided into four groups of six animals in each group. Liver damage was induced by administration of $CCl_4$ (1 ml/kg body weight) intra-peritoneal a day prior to the treatment. Group I received the vehicle (Sodium CMC 0.3%) and served as control and was not treated with the toxicant. The second group served as $CCl_4$ treated control. Group III received a suspension of the total alkaloid of methanolic extract of leaves of *Solanum pseudocapsicum* (20 mg/kg body weight), and group IV received the standard (250 mg/kg body weight). After 24 h of intoxication, the animals received these treatments by the oral route for a period of 6 days. On the 8$^{th}$ day, blood was collected in sterile centrifuge tubes and allowed to clot. Serum was separated and used for the estimation of ASAT, ALAT, ALP, TGL, total proteins, albumin, total bilirubin and direct bilirubin using Ecoline diagnostic kits (Yoshinobu et al., 1983).

### Statistical analysis

Statistical analysis was carried out using Student's *t*-test. The results were judged significant if $P < 0.05$.

## Results

### Hepatoprotective effects in freshly isolated rat hepatocytes

The effects of the total alkaloid fraction of *Solanum pseudocapsicum* on freshly isolated rat hepatocytes intoxicated with $CCl_4$ are recorded in Table 1. A significant increase in the levels of ASAT, ALAT, ALP, total bilirubin, direct bilirubin ($P < 0.001$) and a significant reduction in the levels of TGL, total proteins and albumin ($P < 0.001$) were observed in hepatocytes exposed to $CCl_4$ when compared to normal rats. These cells, when treated along with the total alkaloid fraction of *Solanum pseudocapsicum*, showed a significant restoration of the altered biochemical parameters towards the normal ($P < 0.001$, when compared to $CCl_4$ treated group) and is dose dependent. A similar result was obtained when $CCl_4$ intoxicated hepatocytes were treated with the standard. However, the hepatoprotective effect of total alkaloid of *Solanum pseudocapsicum* was observed at very low concen-

*Table 1.* Effects of treatment of total alkaloid fraction of *Solanum pseudocapsicum* leaves on the biochemical parameters of $CCl_4$ intoxicated freshly isolated rat hepatocytes (number of independent experiments = 3, 5 replicates, mean ± SEM).

| Treatment | Concentration | ASAT U/L | ALAT U/L | ALP U/L | TGL mg/dL | Total Protein g/dL | Albumin g/L | Total Bilirubin mg/dL | Direct Bilirubin mg/dL |
|---|---|---|---|---|---|---|---|---|---|
| Normal | – | 11.00 ± 0.39 | 15.00 ± 0.01 | 27.00 ± 0.46 | 180.00 ± 9.08 | 0.762 ± 0.04 | 1.213 ± 0.06 | 0.204 ± 0.005 | 0.034 ± 0.002 |
| $CCl_4$ | 1% | 69.00 ± 0.48$^{a}$ | 56.00 ± 0.53$^{a}$ | 79.00 ± 2.86$^{a}$ | 97.00 ± 3.06$^{a}$ | 0.107 ± 0.06$^{a}$ | 0.30 ± 0.01$^{a}$ | 0.518 ± 0.01$^{a}$ | 0.174 ± 0.03$^{a}$ |
| $CCl_4$ (1%) + Standard | 250 µg | 20.00 ± 0.83$^{b}$ | 27.30 ± 1.24$^{b}$ | 27.00 ± 0.97$^{b}$ | 190.00 ± 12.47$^{b}$ | 0.65 ± 0.024$^{b}$ | 1.10 ± 0.03$^{b}$ | 0.275 ± 0.02$^{b}$ | 0.059 ± 0.002$^{b}$ |
| $CCl_4$ (1%) + Total alkaloid fraction | 10 µg | 13.00 ± 0.92$^{b,d}$ | 19.00 ± 1.22$^{b,e}$ | 28.80 ± 0.98$^{b}$ | 197.60 ± 10.08$^{b}$ | 0.74 ± 0.02$^{b}$ | 1.20 ± 0.04$^{b}$ | 0.255 ± 0.04$^{b,c}$ | 0.044 ± 0.002$^{b,d}$ |
| | 9 µg | 13.40 ± 0.73$^{b,c}$ | 26.80 ± 0.66$^{b}$ | 26.00 ± 1.39$^{b}$ | 193.20 ± 8.90$^{b}$ | 0.706 ± 0.03$^{b}$ | 1.13 ± 0.07$^{b}$ | 0.27 ± 0.01$^{b}$ | 0.055 ± 0.002$^{b}$ |
| | 8 µg | 19.40 ± 0.51$^{b}$ | 25.80 ± 1.02$^{b}$ | 29.20 ± 1.34$^{b}$ | 186.60 ± 9.81$^{b}$ | 0.62 ± 0.03$^{b}$ | 0.994 ± 0.05$^{b}$ | 0.299 ± 0.02$^{b}$ | 0.06 ± 0.001$^{b}$ |
| | 7 µg | 21.60 ± 0.51$^{b}$ | 26.8 ± 0.99$^{b}$ | 30.20 ± 1.76$^{b}$ | 184.40 ± 5.52$^{b}$ | 0.584 ± 0.02$^{b}$ | 0.912 ± 0.05$^{b}$ | 0.305 ± 0.02$^{b}$ | 0.071 ± 0.003$^{b}$ |
| | 6 µg | 24.80 ± 0.58$^{b}$ | 30.02 ± 1.63$^{b}$ | 33.00 ± 1.80$^{b}$ | 180.00 ± 6.73$^{b}$ | 0.544 ± 0.03$^{b}$ | 0.902 ± 0.04$^{b}$ | 0.359 ± 0.02$^{b}$ | 0.079 ± 0.003$^{b}$ |

a = P < 0.001, when compared to normal group, b = P < 0.001, when compared to $CCl_4$ group, c = P < 0.05, d = P < 0.01, e = P < 0.001, when compared to standard.

trations (6–10 µg/ml) when compared to the standard. The decrease in the levels of ASAT, ALAT, total bilirubin and direct bilirubin in freshly isolated hepatocytes treated with total alkaloid fraction at 10 µg/ml was significant ($P < 0.05 – 0.001$, when compared to standard) and more than that produced by the standard at 250 µg/ml.

### Hepatoprotective effects in the HepG2 cell line

The $CCl_4$ exposed HepG2 cells showed a percentage viability of 24%. These exposed cells, when treated with different concentrations of the total alkaloid fraction of *Solanum pseudocapsicum*, showed a dose-dependent increase in percentage viability and the results were highly significant ($P < 0.001$, when compared to $CCl_4$ intoxicated group). The percentage viability ranged between 77–94% at 6–10 µg/ml concentration of the total alkaloid fraction (Table 2). The increase in percentage viability of the Hep G2 cells treated with total alkaloid fraction at 10 and 9 µg/ml was significant ($P < 0.01$, when compared to standard) and more potent than that produced by the standard at 250 µg/ml.

### *In vivo* hepatoprotective effects

The effects of total alkaloid fraction of *Solanum pseudocapsicum* on $CCl_4$ intoxicated rats are recorded in Table 3. Intoxication of rats treated with $CCl_4$ significantly altered the biochemical parameters when compared with normal control rats ($P < 0.001$). Treatment with total alkaloid fraction of *Solanum pseudocapsicum* at 20 mg/kg body weight showed a significant decrease in ASAT, ALAT, ALP, total bilirubin, direct bilirubin ($P < 0.001$) and a significant elevation in the TGL, total proteins and albumin levels ($P < 0.001$) in serum when compared with $CCl_4$ treated rats. Standard at 250 mg/kg body weight also exhibited similar results.

*Table 2.* Hepatoprotective activity of the total alkaloid fraction of *Solanum pseudocapsicum* leaves on $CCl_4$ intoxicated HepG2 cells.

| Treatment | Concentration (µg/ml) | % Viability |
|---|---|---|
| Control | – | 100 |
| $CCl_4$ | – | $24.39 \pm 2.16^{a}$ |
| $CCl_4$ + Standard | 250 | $80.14 \pm 3.12^{b}$ |
| $CCl_4$ + Total alkaloid fraction | 10 | $93.72 \pm 4.21^{b,c}$ |
| | 9 | $88.85 \pm 3.94^{b,c}$ |
| | 8 | $84.32 \pm 3.65^{b}$ |
| | 7 | $82.23 \pm 4.01^{b}$ |
| | 6 | $77.35 \pm 3.03^{b}$ |

(Average of 5 determinations, 4 replicates).
a = $P < 0.001$, when compared to normal cells.
b = $P < 0.01$, when compared to $CCl_4$ intoxicated cells.
c = $P < 0.01$, when compared to standard treated cells.

*Table 3.* Effects of treatment with the total alkaloid fraction of *Solanum pseudocapsicum* on the biochemical parameters of $CCl_4$ intoxicated rats.

| Treatment | Dose | ASAT U/L | ALAT U/L | ALP U/L | TGL mg/dL | Total Protein g/dL | Albumin g/L | Total Bilirubin mg/dL | Direct Bilirubin mg/dL |
|---|---|---|---|---|---|---|---|---|---|
| Normal | – | 61.69 ± 2.102 | 25.63 ± 0.537 | 257.65 ± 13.55 | 69.40 ± 3.127 | 7.054 ± 0.295 | 3.744 ± 0.22 | 0.408 ± 0.051 | 0.16 ± 0.04 |
| $CCl_4$ | 1 ml/kg body wt | 105.79 ± 4.604[a] | 57.35 ± 2.04[a] | 469.80 ± 12.18[a] | 25.60 ± 1.42[a] | 4.108 ± 0.104[a] | 1.964 ± 0.12[a] | 1.014 ± 0.04[a] | 0.48 ± 0.02[a] |
| $CCl_4$ (1 ml/kg body wt) + Standard | 250 mg/kg body wt | 54.756 ± 3.43[c] | 24.52 ± 1.47[c] | 316.00 ± 10.97[c] | 59.00 ± 2.47[c] | 6.96 ± 0.324[c] | 3.89 ± 0.09[b] | 0.39 ± 0.012[c] | 0.17 ± 0.01[c] |
| $CCl_4$ (1 ml/kg body wt) + Total alkaloid fraction | 20 mg/kg body wt | 51.66 ± 2.394[c] | 30.029 ± 1.29[c] | 344.40 ± 12.41[c] | 51.00 ± 2.61[c] | 6.22 ± 0.208[b] | 3.516 ± 0.17[b] | 0.444 ± 0.104[c] | 0.22 ± 0.013[c] |

a = $P < 0.001$, when compared to normal group, b = $P < 0.01$, c = $P < 0.001$, when compared to $CCl_4$ group.

## Discussion

The present study reveals the hepatoprotective effect of *Solanum pseudocapsicum* against $CCl_4$ induced toxicity in isolated rat hepatocytes, HepG2 cells in culture and in animal models. $CCl_4$ has been found to induce extensive liver damage within a period of 24 h following intra-peritoneal administration. As a result of this, accumulation of fat in the liver and necrosis in the centrilobular region of the liver occurs. As a consequence, the microsomal enzyme activities are found to decrease and due to lipid peroxidation, the water-soluble enzymes leak into plasma from the liver. It is shown by the significant decrease in triglycerides and proteins in $CCl_4$ intoxicated rat hepatocytes or animals in the present studies. Treatment with the total alkaloid fraction of *Solanum pseudocapsicum* exhibited significant restoration of the altered biochemical parameters towards normal in $CCl_4$ intoxicated rat hepatocytes and in rats. The effect of the total alkaloid at 10 µg/ml was found to be better that that of standard at 250 µg/ml. Its hepatoprotective effect with *in vivo* studies at 20 mg/kg body weight was comparable to that of standard at 250 mg/kg body weight. The investigation carried out in human liver derived HepG2 cells against $CCl_4$ induced damage also confirmed the hepatoprotective nature of the total alkaloid fraction.

Several plants belonging to the genus *Solanum* have also exhibited hepatoprotective effects. An aqueous extract of *Solanum alatum* Moench. (Solanaceae) at 100 and 200 mg/kg body weight exhibited hepatoprotective activity against $CCl_4$ induced liver injury in rats (Lin et al., 1995). The plant *Solanum nigrum* Linn. (Solanaceae) also possessed strong hepatoprotective effects (Sultana et al., 1995). The triterpenoid daturalone isolated from *Solanum arundo* Juss. (Solanaceae) showed potent hepatoprotective effect in both acute and chronic liver damage induced in rats (Grace & Salen, 1996). Several steroidal alkaloids isolated from *Solanum* species inhibited hepatotoxicity in mice (Gan et al., 1993). Solasodin (3 mg/kg body weight), solamargine (1 mg/kg body weight), solasonine (0.1 mg/kg body weight), ursolic acid (10 mg/kg body weight) and carpesterol (10 mg/kg body weight) isolated from *Solanum incanum* Linn. (Solanaceae) exhibited strong hepatoprotective activity against liver damage induced by $CCl_4$ (Chun et al., 1987). Capsimine and isocapsicastrine, two steroidal alkaloids isolated from *Solanum capsicastrum* Linn. (Solanaceae) exhibited strong hepatoprotective effects (Chun & Kim, 1989). Several steroidal alkaloids, namely solanocapsine, solacasine, solacapine, episolacapine, isosolacapine and solasodine, have been isolated from the whole plant of *Solanum pseudocapsicum* (Chakravarty et al., 1984). Hence, the hepatoprotective effect observed in the present study may be mainly due to the presence of any of these steroidal alkaloids present in the total alkaloid fraction of the leaves of *Solanum pseudocapsicum*. The results from the present study indicate a good correlation between the *in vivo* and *in vitro* studies. In conclusion, the total alkaloid fraction merits further investigation in identifying the active constituents responsible for this activity.

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