# Antiallergic, Antipyretic, Hypoglycemic and Hepatoprotective Effects of Aqueous Extract of *Coronopus didymus* Linn

Sudheer Kumar Mantena,* Srinivasa Mutalik, Hullegala Srinivasa, Ganesa Sundararajan Subramanian, Koiram Rajanna Prabhakar, Kasu Raghurami Reddy, Keloth Kaitheri Srinivasan, and Mazhuvancherry Kesavan Unnikrishnan

*Manipal College of Pharmaceutical Sciences; Manipal-576104, Karnataka, India.*
Received September 10, 2004; accepted November 22, 2004

**The aqueous extract of whole plant of *Coronopus didymus* Linn (CD) [Family: Bracicacea] was screened for antiallergic, antipyretic and hepatoprotective effects in rats and hypoglycemic activity in mice. The extract showed significant antiallergic, antipyretic, hypoglycemic and hepatoprotective activity at 200 and 400 mg/kg doses on oral administration. Mechanistically, CD acts as an antioxidant as evidenced by its ability to scavenge DPPH and superoxide radicals. All the observed activities may be due to the presence of flavonoids, saponins and tannins as they are reported to possess a variety of biological activities.**

**Key words** *Coronopus didymus*; antiallergic; antipyretic; hypoglycemic; hepatoprotective

The search for new pharmacologically active agents obtained by screening natural sources such as plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. Based on the ethnobotanical communication, *Coronopus didymus* Linn (Family: Bracicacea) was selected for the study. *Coronopus didymus* (CD) has been in use as folk medicine to treat allergies and wounds in Karnataka, India. CD has been reported to contain benzyl cyanide,[1] glucotrapaeolin, a glucoside of benzyl isothiocyanate,[2] 1,8-dihyroxy anthraquinone at a concentration of 0.003%[3] and flavones, chrysoeriol and chrysoeriol-6″(OAc)-4′-$\beta$-D-glucoside.[4] We have previously reported the wound healing and anti-inflammatory activities of ethanolic and aqueous extracts of CD and aqueous extract was found to be more active.[4] In the present study, the aqueous extract of CD has been evaluated for antiallergic, antipyretic and hepatoprotective effects in rats and hypoglycemic activity in mice.

## MATERIALS AND METHODS

**Plant Material** The whole plant of CD was collected from Chickballapur, Karnataka, India, in May, identified and authenticated by Dr. Gopalakrishna Bhat, Professor, Department of Botany, Poorna Prajna College, Udupi, India. A voucher specimen of the plant is deposited in the Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Manipal, India.

**Chemicals** Compound 48/80, RPMI-1640, streptozotocin, xanthine, xanthine oxidase, nitroblue tetrazolium and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Inc., U.S.A. Triple antigen was obtained from Biological E. Ltd., Hyderabad, India. Glibenclamide and Ketotifen fumarate were obtained from Microlabs Ltd., Bangalore, India. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were of Enzopak kits, Reckon Diagnostics, Vadodara. The sheep serum was prepared from the fresh blood collected from the slaughterhouse under sterile condition. All the other chemicals used were of analytical grade.

**Animals** Wistar rats (weighing 150—200 g) and Swiss albino mice (weighing 25—30 g) were obtained from Central Animal House, Department of Pharmacology, Kasturba Medical College, Manipal. The animals were kept at 25±1 °C and 45—55% relative humidity with a 12 h light/dark cycle. They were fed with standard laboratory diet (Lipton Rat Feed, Bombay, India) and water.

**Preparation of Aqueous Extract** The aqueous extract of CD was prepared by boiling about 200 g of the plant in water (1 l), and concentrated *in vaccuo* to a semisolid consistency (yield: 20 g). The extract was subjected to various phytochemical tests. The extract on suspension in methanol yielded a solid material, which was filtered out (compound $C_1$). The filterate was subjected to paper chromatography (Whatmann No. 1; ascending (25±2 °C); 15% acetic acid; visualization: exposure to ammonia vapour).

**Characterization of Extract by HPLC** The separation was carried out on a reverse phase column (RP C-18 column; Luna, Phenomenex, U.S.A.; 250 mm×4.6 mm; particle size 5 $\mu$m) using gradient elution (Hossinzadeh *et al.*, 2002). Gradient was performed using 0.3% triethanolamine pH 3.0 and acetonitrile at a total flow rate of 1.0 ml/min; gradient composition (min, % acetonitrile): 0, 10; 0.01, 10; 3, 10; 7, 20; 10, 50; 12, 50; 13, 90; 20, 100; 25, 100; 30, 10; 35, 10. The extract was dissolved in distilled water and the eluent was monitored at 270 nm.

**Anti-allergic Activity** a. Sheep Serum Induced Mast Cell Degranulation Method: Wistar rats were sensitized by injecting subcutaneously 0.5 ml of sheep serum along with 0.5 ml of triple antigen containing 20000 million *Bordetella pertusis* organisms.[5] The sensitized rats were divided into 4 groups of 6 animals. Group I served as control and received double distilled water (DDW) orally. Group II served as standard and received ketotifen fumarate 1 mg/kg/d orally. Group III and IV received aqueous extract of CD (200 and 400 mg/kg/d orally) respectively. The treatment was continued for 14 d. During the course of treatment the animals were maintained under the controlled conditions of temperature, and were fed with standard diet and water given *ad libitum*. On the 14th day, 2 h after the assigned treatment, the rats were sacrificed and the intestinal mesentery was taken for studies on the mast cells.

* To whom correspondence should be addressed. e-mail: sudheerkumarm99@yahoo.com

The mesenteries of the sacrificed rats along with pieces of intestine were kept in Ringer-Lock solution at 37 °C (NaCl, 9.0 g; KCl, 0.42 g; $NaHCO_3$, 0.15 g; Glucose, 1.0 g/l of distilled water). The mesenteric pieces were challenged with 5% sheep serum for 10 min and then transferred to a wide mouthed bottle containing 10% formalin. The mesenteric fans were fixed, dried and stained with thionin (0.25%) on a clean slide. The excess stain was washed with distilled water followed by dehydration in absolute alcohol. Finally the slides were cleared in xylene and mounted in diphenylpthalein xylene and examined microscopically for the number of intact and degranulated mast cells in at least ten randomly selected high power fields.

b. Compound 48/80 Induced Mast Cell Degranulation Method: Wistar rats were divided into 5 groups of 6 animals. Group I served as control and received DDW by oral route. Group II served as standard and received ketotifen fumarate 1 mg/kg/d orally. Group III and IV received aqueous extract of CD (200, 400 mg/kg/d orally) respectively, for 7 d. On day 7, two hours after the assigned treatment the peritoneal fluid was collected and the mast cells were isolated as described below.[6]

The rats were anaesthetized with ether and 10 ml normal saline solution was injected into the peritoneal cavity and the abdomen was gently massaged for 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal mast cells was aspirated and collected in siliconised test tubes containing 7—10 ml of RPMI-1640 medium (pH 7.2—7.4). The mast cells were then washed thrice by centrifugation at low speed (400—500 g) and the pellets of mast cells were taken in the medium. The mast cells suspension (approximately $1\times10^6$ cells/ml) was challenged with 1 $\mu$g/ml of compound 48/80 and stained with 1% toluidine blue and observed under high power microscopic field (450×). The number of intact and degranulated cells was counted at least in ten different randomly selected high power fields.

**Antipyretic Activity** The rectal temperature of rats 1 h after 18 h starvation (*i.e.*, at 19th hour after starting the starvation) was recorded and animals having temperature between 37.5 °C and 38.5 °C were selected for the test. The animals were injected intramuscularly with 1 ml of 20% suspension of Brewer's yeast in 0.9% saline.[7] The animals developing 0.5 °C or more rise in the rectal temperature 18 h after injection were divided into 4 groups of 6 animals each. One group served as control and other groups received paracetamol (33 mg/kg) and aqueous extract of CD (200, 400 mg/kg). The rectal temperature was recorded at 0, 1, 2 and 3 h after administration of the test drugs and compared. Percentage reduction in rectal temperature was calculated using following formula.[8] The total fall in rectal temperature from the elevated to normal level was considered as 100%.

$$\text{percentage reduction} = \frac{\text{elevated temperature} - \text{temperature at different interval}}{\text{elevated temperature} - \text{initial temperature}} \times 100$$

**Hypoglycemic Activity** a. In normal Mice: The mice were divided into 4 groups of 6 each after an overnight fast. Blood was collected (0 h) from retro-orbital venus plexus and the aqueous extract of CD (200, 400 mg/kg) and glibenclamide (50 mg/kg) were fed to these mice through a metal canula attached to a hypodermic syringe. Control group received DDW. Blood samples were collected 2, 4, 6 and 10 h after the administration of drugs.[9] The estimation of glucose was done using a glucometer (Accutrend Alpha, Borrheinger Mannham, U.S.A.).

b. In Diabetic Mice: The mice were made diabetic by a single intraperitoneal injection of streptozotocin (150 mg/kg). The fasting glucose level was determined 72 h post-injection. After starvation for 18 h, mice with blood glucose level above 300 mg/dl (0 h) were randomly divided into 4 groups of 6 animals each. Groups 1 and 2 received 200 and 400 mg/kg of aqueous extract of CD orally. Group 3 was given the reference drug glibenclamide; Group 4 received 0.2 ml of DDW. Blood samples were drawn from retro-orbital venus plexus after 2, 4, 6 and 10 h of treatment for the determination of blood levels of glucose.[9]

**Hepatoprotective Activity** The rats were divided into 5 groups of 6 animals each. Group I and II were given DDW. Groups III, IV and V were treated orally with 50 mg/kg of silymarin, 200 and 400mg/kg of aqueous extract of CD respectively for six consecutive days. On the fifth day, except group I, all other groups were given $CCl_4$ (2 ml/kg, in 50% dilution with olive oil) and group I was given equivalent of olive oil only. On seventh day (*i.e.* after 48 h of $CCl_4$ administration), blood was collected from retro-orbital venus plexus and the plasma was separated by centrifuging the blood at 3000 ***g*** for 10 min. Plasma was analysed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels using Autoanalyzer (Hitachi-911, Japan). The rats were sacrificed and liver was taken out. The homogenate of liver (10%) was prepared in ice cold KCl solution (1.15% w/v) using a Teflon homogeniser. A part of the homogenate was used for estimation of glutathione.[10] The remaining homogenate was centrifuged at 4000 ***g*** for 10 min to remove nuclear fraction. The supernatant was used for the estimation of lipid peroxide[11] and total protein levels.[12]

**Free Radical Scavenging Activity** a. Scavenging of DPPH Radical: To the ethanolic solution of DPPH (0.05 mM) an equal volume of the aqueous extract of CD in phosphate buffer (pH 7.4, 20 mM) was added at various concentrations (10—250 $\mu$g/ml) in a final volume of 1.0 ml. An equal amount of phosphate buffer was added to control. After 20 min, absorbance was recorded at 517 nm.[13]

b. Scavenging of Superoxide Radical: Superoxide was generated using xanthine–xanthine oxidase system and measured by reduction of nitroblue tetrazolium. The reaction mixture containing various concentrations (10—250 $\mu$g/ml) of extract, xanthine oxidase (0.001 units), nitroblue tetrazolium (300 $\mu$M), xanthine (200 $\mu$M) and phosphate buffer (pH 7.8, 20 mM) in a final volume of 1.0 ml was incubated at 25±1 °C for 20 min. The absorbance was measured at 560 nm.[4] The difference in absorbance between test and control was taken and expressed as % scavenging of superoxide radical.

**Statistical Analysis** The statistical evaluation of the data was done by ANOVA using Graph PAD Instat Software and $p$ value less than 0.05 was considered to be significant.

## RESULTS

**Chemistry of Aqueous Extract** The compound $C_1$ was identified as potassium nitrate on systematic qualitative inor-

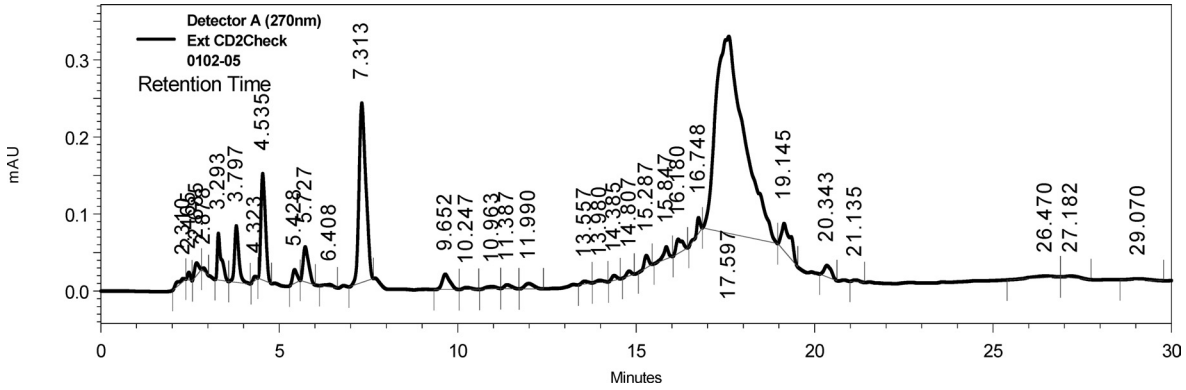


Fig. 1. HPLC Chromatogram of the Aqueous Extract of CD

Column: Reverse Phase column (RP C-18 column; Luna, Phenomenex, U.S.A.; 250 mm×4.6 mm; particle size 5 $\mu$m). Gradient elution: 0.3% triethanolamine pH 3.0 and acetonitrile at a total flow rate of 1.0 ml/min. Detection: 270 nm.

ganic analysis. The filtrate from aqueous extract on paper chromatography gave a yellow spot on exposure to ammonia almost at the solvent front, which confirms the presence of a flavonoid glycoside [Whatmann No. 1; ascending (±25 °C); 15% acetic acid; visualization: exposure to ammonia vapour]. The structure of this is yet to confirmed by the spectral data for which further studies are necessary. Other preliminary investigations on the extract showed the presence of saponins and tannins. The HPLC fingerprints of the extract are shown in Fig. 1. Prominent peaks were observed at retention time of 5.727, 7.31 and 17.59 min with area % of 1214566, 3086184 and 14131514 respectively.

**Anti-allergic Activity** Effects of CD on mast cell degranulation induced by sheep serum and compound 48/80 are shown in Table 1. There was a significant dose dependent protection against mast cell degranulation with aqueous extract of CD. The percentage of intact mast cells observed for aqueous extract (400 mg/kg) was 63.79±1.22 and 61.36± 3.09 against sheep serum and compound 48/80 respectively.

**Antipyretic Activity** The results of effect of aqueous extract of CD on yeast induced pyrexia in rats are depicted in Table 2. The extract produced significant antipyretic effect in a dose dependent manner. The antipyretic effect noticed at a dose of 400 mg/kg was comparable to paracetamol.

**Hypoglycemic Activity** The results of hypoglycemic effect, in normal as well as in streptozotocin induced diabetic mice, of aqueous extract of CD are shown in Tables 3 and 4, respectively. Streptozotocin produced moderate to severe hyperglycemia in mice (300—600 mg/dl). The animals with 300—500 mg/dl blood glucose levels were used for the antihyperglycemic study. The extract produced a significant hypoglycemic and antihyperglycemic activity in dose dependent manner, comparable to glibenclamide.

**Hepatoprotective Activity** As shown in Tables 5 and 6, there was a drastic impairment in the hepatic functions after 48 h of $CCl_4$ administration, which was indicated by elevation in plasma AST, ALT and hepatic lipid peroxide levels. Aqueous extract of CD (200, 400 mg/kg) significantly reversed these changes and was comparable to that of silymarin. It produced a significant increase in the protein and GSH levels and decreased the lipid peroxide levels compared to $CCl_4$ control.

**Free Radical Scavenging Activity** Table 7 shows the effect of CD in scavenging DPPH and superoxide radicals, respectively. CD scavenged the DPPH radical in a concentration dependent manner (10—250 $\mu$g/ml), while the scavenging of superoxide reached saturation at a concentration of 150 $\mu$g/ml.

Table 1. Effect of Aqueous Extract of CD on Mast Cell Degranulation Induced by Sheep Serum and Compound 48/80

| Treatment | Dose (mg/kg) | Intact mast cells (%) | |
|---|---|---|---|
| | | Sheep serum | Compound 48/80 |
| Gum acacia (control) | 2% | 23.38±1.29 | 20.08±0.49 |
| Ketotifen fumerate | 1 | 74.31±3.38[b] | 71.59±1.84[b] |
| CD | 200 | 56.34±2.16[b,d] | 50.84±3.85[b,d] |
| CD | 400 | 63.79±1.22[b,c] | 61.36±3.09[b] |

All the values are expressed as mean±S.E. ($n$=6); (b) $p<0.001$, compared to control; (c) $p<0.05$; (d) $p<0.01$, compared to ketotifen fumerate treated group.

## DISCUSSION

In our earlier study,[4] the animals showed good tolerance to aqueous extract of CD and a dose as high as 2 g/kg was found to be lethal. Hence 200 and 400 mg/kg doses (1/10 and 1/5th of lethal dose) were selected in the present study. The preliminary phytochemical screening showed the presence of flavonoid glycoside, saponins and tannins. The isolated compound, $C_1$, was identified as potassium nitrate.

In the antiallergic activity, aqueous extract of CD was found to inhibit the degranulation of mast cells induced by non-immunological and immunological stimulus. Agents (Compound 48/80), which cause the release of mediators, from mast cells, act by alteration of the cell membrane structure leading to increase in permeability and consequent release of mediators from the cell granules.[15] The extract may act to stablilize the membrane and, hence, inhibit the degranulation of mast cells. The flavonoids, kaempferol and quercetin, have been proved to be antiallergic compounds.[16] Prabhakar *et al.*[4] reported the presence of two flavonoids, chrysoeriol and chrysoeriol-6″(OAc)-4′-$\beta$-D-glucoside in the ethyl acetate fraction of CD. Also, chemistry of aqueous extract showed the presence of flavonoid glycoside. It is quite possible that these components may contribute to the anti-allergic activity manifested.

Aqueous extract of CD produced significant antipyretic ef-

Table 2. Effect of Aqueous Extract of CD on Yeast Induced Pyrexia in Rats

| Treatment | Initial temp. (°C) | Temp. after 18 h of yeast admn. (°C) | Temp. in °C (mean±S.E) | | | % Reduction in temp. (mean±S.E) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 h | 2 h | 3 h | 1 h | 2 h | 3 h |
| Control | 37.97±0.15 | 38.70±0.15 | 38.67±0.14 | 38.67±0.14 | 38.67±0.16 | 2.8±2.77 | 2.8±2.77 | 2.9±1.36 |
| Paracetamol (33 mg/kg) | 37.65±0.15 | 38.40±0.11 | 37.95±0.18 | 37.83±0.14 | 37.05±0.23 | 58.9±14.78[b,d] | 75.6±5.17[c,f] | 89.5±0.16[c,f] |
| CD (200 mg/kg) | 37.45±0.09 | 38.37±0.14 | 38.26±0.11 | 38.12±0.10 | 37.95±0.09 | 10.1±3.72 | 26.8±3.67 | 46.5±6.15[c] |
| CD (400 mg/kg) | 37.83±0.13 | 38.45±0.13 | 38.25±0.12 | 38.05±0.12 | 38.00±0.10 | 24.9±5.92 | 65.8±9.61[c,e] | 69.4±7.19[c,d] |

All the values are expressed as mean±S.E. ($n$=6); (b) $p<0.01$; (c) $p<0.001$ compared to control; (d) $p<0.05$; (e) $p<0.01$; (f) $p<0.001$ compared to aqueous extract of CD (200 mg/kg) treated group.

Table 3. Effect of Aqueous Extract of CD on the Blood Glucose Level of Fasted Swiss Albino Mice

| Treatment | Blood glucose levels (mg/dl) | | | | |
|---|---|---|---|---|---|
| | 0 h | 2 h | 4 h | 6 h | 10 h |
| Normal | 155.0±5.77 | 151.2±6.08 (2.6±0.44) | 151.6±5.54 (2.6±0.33) | 153.5±7.04 (1.1±0.83) | 151.3±5.81 (2.4±0.44) |
| Glibenclamide | 121.0±4.65 | 72.0±4.65 (40.2±1.30)[b] | 70.0±3.27 (42.2±0.61)[b] | 67.3±3.44 (44.4±0.89)[b] | 65.2±3.11 (46.0±0.71)[b,f] |
| CD (200 mg/kg) | 167.7±12.25 | 126.0±5.85 (24.9±0.51)[b] | 100.0±6.51 (40.4±0.49)[b] | 95.0±6.0 (43.3±0.75)[b] | 108.0±5.13 (35.6±0.48)[b] |
| CD (400 mg/kg) | 210.0±12.50 | 125.2±7.21 (40.4±0.82)[b,f] | 107.0±10.41 (49.1±2.01)[b,d,e] | 101.0±12.34 (51.6±1.98)[b,c,e] | 109.0±11.13 (48.1±2.12)[b,f] |

All the values are expressed as mean±S.E. ($n$=6); (b) $p<0.001$ compared to control; (c) $p<0.05$; (d) $p<0.01$ compared to glibenclamide treated group; (e) $p<0.01$; (f) $p<0.001$ compared to aqueous extract of CD (200 mg/kg) treated group. The values given in parenthesis represent the percentage reduction in blood glucose levels.

Table 4. Effect of Aqueous Extract of CD on the Blood Glucose Level of Steptozotocin Induced Diabetic Swiss Albino Mice

| Treatment | Blood glucose levels (mg/dl) | | | | |
|---|---|---|---|---|---|
| | 0 h | 2 h | 4 h | 6 h | 10 h |
| Normal | 341.0±6.39 | 334.0±4.69 (2.1±0.51) | 333.2±4.33 (2.1±0.75) | 331.3±6.79 (2.7±1.0) | 330.0±5.27 (2.92±0.8) |
| Glibenclamide | 320.0±5.81 | 214.0±3.57 (33.1±0.72)[b,g] | 203.0±3.24 (36.2±1.47)[b,g] | 200.4±2.91 (37.5±1.0)[b] | 198.0±2.96 (37.9±0.7)[b] |
| CD (200 mg/kg) | 460.0±21.16 | 431.0±11.50 (6.3±1.8) | 364.0±13.07 (20.9±2.0)[b] | 262.3±9.35 (42.9±1.0)[b,c] | 310.0±11.01 (32.6±2.1)[b] |
| CD (400 mg/kg) | 327.0±12.34 | 185.3±8.37 (43.3±1.9)[b,d,g] | 175.7±9.61 (46.4±1.3)[b,d,g] | 165.7±5.21 (49.3±1.2)[b,e,f] | 209.0±6.62 (46.1±1.6)[b,c,f] |

All the values are expressed as mean±S.E. ($n$=6); (b) $p<0.001$ compared to control; (c) $p<0.05$; (d) $p<0.01$; (e) $p<0.001$ compared to glibenclamide treated group; (f) $p<0.01$; (g) $p<0.001$ compared to aqueous extract of CD (200 mg/kg) treated group. The values given in parenthesis represent the percentage reduction in blood glucose levels.

Table 5. Effect of Aqueous Extract of CD on $CCl_4$ Induced Elevation in Plasma Transaminases

| Treatment | Hepatocellular injury | |
|---|---|---|
| | AST (IU) | ALT (IU) |
| Vehicle | 52.7±1.67 | 35.7±2.13 |
| $CCl_4$ | 81.1±1.78[b] | 187.3±4.52[c] |
| Silymarin+$CCl_4$ | 54.9±4.83[d] | 47.3±4.05[e] |
| CD (200 mg/kg)+$CCl_4$ | 64.0±1.98 | 116.6±3.18[e,f] |
| CD (400 mg/kg)+$CCl_4$ | 56.5±2.52[d] | 51.4±2.10[e,f,g] |

All values are mean±S.E. ($n$=6). (b) $p<0.01$, (c) $p<0.001$ compared to vehicle control; (d) $p<0.01$, (e) $p<0.001$ compared to $CCl_4$ treated group; (f) $p<0.001$ compared to silymarin treated group; (g) $p<0.001$ compared to aqueous extract (200 mg/kg) treated group.

Table 6. Effect of Aqueous Extract of CD on Changes in $CCl_4$ Induced Alterations in Functional and Antioxidant Status of Rat Liver

| Treatment | Total proteins (mg/g of tissue) | Lipid peroxides (nmol MDA/100 g protein) | GSH ($\mu$M/g of tissue) |
|---|---|---|---|
| Vehicle | 130.82±5.19 | 409.48±14.07 | 9.7±0.46 |
| $CCl_4$ | 84.80±1.69[d] | 808.23±25.18[d] | 4.6±0.72[d] |
| Silymarin+$CCl_4$ | 114.08±3.96[b,g] | 557.32±26.17[f] | 7.2±0.04[b,e] |
| CD 200 mg/kg+$CCl_4$ | 95.31±3.61[d] | 682.25±19.95[d,h] | 6.1±0.25[c] |
| CD 400 mg/kg+$CCl_4$ | 105.23±1.17[b,g] | 562.42±34.39[c,e] | 6.9±0.31[c,e] |

All values are mean±S.E. ($n$=6). (b) $p<0.05$, (c) $p<0.01$, (d) $p<0.001$ compared to vehicle control; (e) $p<0.05$, (f) $p<0.01$, (g) $p<0.001$ compared to $CCl_4$ treated group; (h) $p<0.05$ compared to silymarin treated group.

Table 7. Interaction of CD with DPPH and Superoxide Radicals

| CD conc. ($\mu$g/ml) | DPPH scavenging | | Superoxide scavenging | |
|---|---|---|---|---|
| | Absorbance ±S.E. | % Scavenging | Absorbance ±S.E. | % Scavenging |
| Control | 0.771±0.005 | — | 0.466±0.006 | — |
| 250 | 0.345±0.012[c] | 55.19 | 0.283±0.002[c] | 39.24 |
| 150 | 0.493±0.005[c] | 35.99 | 0.273±0.005[c] | 41.39 |
| 50 | 0.577±0.011[c] | 25.13 | 0.320±0.001[c] | 31.45 |
| 30 | 0.680±0.003[b] | 11.81 | 0.362±0.004[c] | 22.44 |
| 10 | 0.740±0.004 | 3.94 | 0.424±0.003[b] | 9.01 |

All values are mean±S.E. ($n$=3). (b) $p<0.05$, (c) $p<0.001$ compared to control.

fect in a dose dependent manner. Flavonoids present in the extract may be responsible for antipyretic effect as they have been reported to reduce increased body temperature in many earlier studies.[17,18] An earlier study reported that an increase in the body temperature is associated with elevated lipid peroxide levels, suggesting that pyrexia is associated with increased oxidative stress. They also reported that antioxidant supplementation decreased the lipid peroxidation processes,[19] thereby reducing the body temperature. In the present study, the extract showed significant antioxidant activity, which may be one of the possible mechanisms for observed antipyretic property of CD (Tables 6, 7).

The extract showed significant hypoglycemic activity in normal and diabetic mice in a dose dependent manner. This may be due to the presence of various constituents like saponins, flavonoids and tannins. In many studies the hypoglycemic effect of these agents is already well documented. They have been reported to potentiate plasma insulin effect by increasing the pancreatic secretion of insulin from $\beta$-cells[20—22] and/or may increase the peripheral uptake of glucose.[23] The extract also exhibited significant free radical scavenging activity, as evidenced by its ability to react with DPPH and superoxide radicals (Table 7). Free radicals are generated under various pathological conditions including hyperglycemia and hence diabetes is generally associated with increased oxidative stress.[24,25] The present results demonstrate the antioxidant activity of the plant may also be responsible for the antidiabetic activity. However further studies are necessary to find out the exact mechanism.

Hepatotoxin ($CCl_4$) produced marked liver damage, which was evidenced by substantial enzymatic changes in serum and liver samples. CD (200, 400 mg/kg) significantly reversed the $CCl_4$ induced changes, and the results were comparable to that of silymarin (standard). The hepatoxin elevated levels of AST, ALT and hepatic lipid peroxides were markedly reduced and depleted hepatic GSH levels were partly restored by the extract. The ability of CD to show significant protection against lipid peroxidation indicates a strong antioxidant property.

It is a well documented fact that most medicinal plants are enriched with bioflavonoids, which have antioxidant activity. CD is one such plant containing bioactive flavonoids[4] which are reported free radical scavengers and antioxidants.[26] Flavonoids as antioxidants exhibited several biological effects such as anti-hepatotoxic, anti-inflammatory, antiallergic, antidiabetic and antipyretic actions.[16]

In conclusion, the aqueous extract of whole plant of *Coronopus didymus* Linn exhibited significant antiallergic, antipyretic, hypoglycemic and hepatoprotective activities in animal model. The presence of flavonoids, saponins and tannins in CD might be responsible for the biological activities of the extract in this study.

**Acknowledgements** We are grateful to Ms. Chetana and Ms. Sulochana for their help and cooperation.

REFERENCES

1) Inam-ul-haque, *J. Chem. Soc. Pakistan*, **11**, 80—81 (1989).
2) McDowall F. H., Mortan I. D., McDowell A. K. R., *New Zealand J. Sci. Tech.*, **A28**, 305—307 (1947).
3) De Ruiz R. E. L., Fusco M., Sosa A., Ruiz S. O., *Fitoterpia*, **65**, 181—182 (1994).
4) Prabhakar K. R., Srinivasan K. K., Padma G. M. R., *Pharm. Biol.*, **40**, 490—493 (2002).
5) Gupta S. S., Tripathi R. M., *Aspects. Allergy. Applied. Immunol.*, **6**, 93—99 (1973).
6) Salil S., Gupta P. P., Rajendra P., Dixit K. S., Palit G., Ali B., Gopal M., Saxena R. C., *Indian J. Physiol. Phamacol.*, **43**, 235—241 (1999).
7) Rao R. R., Babu R. M., Rao M. R. V., Babu M. G. V., *Indian Drugs*, **34**, 690—693 (1997).
8) Mutalik S., Paridhavi K., Mallikarjuna Rao C., Udupa N., *Indian J. Pharmacol.*, **5**, 312—315 (2003).
9) Grover J. K., Vats V., Rathi S. S., Dawar R., *J. Ethnopharmacol.*, **76**, 233—238 (2001).
10) Sedlak J., Lindsay R. H., *Analytical Biochem.*, **25**, 192—205 (1968).
11) Ohkawa H., Ohishi N., Yagi K., *Analytical Biochem.*, **95**, 351—358 (1979).
12) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 265—275 (1951).
13) Mantena S. K., Jagadish P., Badduri S. R., Siripurapu K. B., Unnikrishnan M. K., *Nahrung*, **2**, 126—131 (2003).
14) Sreejayan N., Rao M. N. A., *Fitoterpia*, **LXII**, 344—346 (1990).
15) Schwartz L. B., *Curr. Opin. Immunol.*, **6**, 91—94 (1994).
16) Rajanarayana K., Sripal Reddy M., Chaluvadi M. R., Krishna D. R., *Indian J. Pharmacol.*, **33**, 2—16 (2001).
17) Brasseur T., *J. Pharm. Belg.*, 235—241 (1989).
18) Vimala R., Nagarajan S., Alam, Susant J., *Indian J. Exptl. Biol.*, **35**, 1310—1314 (1990).
19) Brzezinska S. E., *J. Physiol. Pharmacol.*, **52**, 275—284 (2001).
20) Pari L., Ramakrishnan R., Venkateshwaran S., *J. Pharm. Pharmacol.*, **53**, 1139—1143 (2001).
21) Achrekar S., Kaklij G. S., Pote M. S., Kelkar S. M., *In Vivo*, **5**, 143—147 (1991).
22) Yanardag R., Colak H., *Pharm. Pharmacol. Commun.*, **4**, 309—311 (1998).
23) Bajaj S., Srinivasan B. P., *Indian J. Pharmacol.*, **31**, 138—141 (1999).
24) Cai L., Kang Y. J., *Cardio. Toxicol.*, **1**, 181—193 (2001).
25) Kumari K., Augusti K. T., *Indian J. Exptl. Biol.*, **40**, 1005—1009 (2002).
26) Mishra B., Priyadarsini K. I., Mantena S. K., Unnikrishnan M. K., Mohan H., *Bioorg. Med. Chem.*, **11**, 2677—2685 (2003).