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Hepatoprotective activity of two plants belonging to the Apiaceae and the Euphorbiaceae family

Bahar Ahmed *, Tanveer Alam, Manoj Varshney, Shah Alam Khan

Department of Pharmaceutical Chemistry, Antihepatotoxic Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110 062, India

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Abstract

The different extracts of *Apium graveolens* Linn. (Apiaceae) and *Croton oblongifolius* Roxb. (Euphorbiaceae) were tested for their hepatoprotective activity against CCl_4 induced hepatotoxicity in albino rats. The degree of protection was measured by using biochemical parameters like serum transaminases (SGOT and SGPT), alkaline phosphatase, total protein and albumin. The methanolic extracts showed the most significant hepatoprotective activity comparable with standard drug silymarin. Other extracts namely petroleum ether and acetone also exhibited a potent activity.

Keywords: *Apium graveolens*; *Croton oblongifolius*; Hepatoprotective activity; Silymarin; CCl_4

1. Introduction

Apium graveolens Linn. (Apiaceae) grows widely in the foothills of North-Western Himalayas and the outlying hills of Punjab, Himachal Pradesh and Uttar Pradesh. It is commonly known as 'Ajmod' and the fruits are popularly known as Celery seeds. As antispasmodic, they are used in bronchitis, asthma and to some extent for liver and spleen diseases (Nadkarni and Nadkarni, 1976; Singh and Handa, 1995).

Croton oblongifolius Roxb., popularly known as 'Chucka' in Hindi, is middle-sized tree belonging to the family Euphorbiaceae. It grows widely in India (Kirtikar and Basu, 1997). Bark is used in reducing chronic enlargement of the liver and in remittent fever. It is applied externally to the hepatic region in chronic hepatitis (Nadkarni and Nadkarni, 1954). The (50%) ethanolic extract of the aerial parts shows hypotensive activity (Bhakuni et al., 1971).

Polyherbal formulations reputed to have hepatoprotective activity that are available on the Indian market comprise about 100 Indian Medicinal Plants (Handa et al., 1986). Reports on the hepatoprotective activity of the following species have been published: *Capparis spinosa* (Shirwaiker et al., 1996), *Daccus carota* (Bhayee et al., 1995), *Euphorbia antisyphilitica* (Saraf et al., 1996), *Hygrophyla auriculata* (Singh and Handa, 1995), *Lycium chinensis* (Kim et al., 1994), *Rubia cordifolia* (Gilani and Janbaz, 1995), *Silybum marianum* (Wang et al., 1996), *Zingiber officinale* (Aggarwal and Prakash, 1995).

The present pharmacological investigation focuses on evaluation of the efficacy of different extracts of seeds of *A. graveolens* and aerial parts of *C. oblongifolius* for their protection against CCl_4- induced hepatotoxicity.

2. Materials and methods

2.1. Plant material

Seeds of *A. graveolens* and aerial parts of *C. oblongifolius* were procured from the Herba Indica, Chandigarh, India, in the month of May 1998 and authenticated by a taxonomist Dr H.S. Puri, the Director of Herba Indica. The voucher specimens (collection nos. 715 and 125) are kept in the herbarium of Jamia Hamdard for future reference.

* Corresponding author. Tel.: +91-11-6088682x354, 352 (O)/6081791 (R); fax: +91-11-6088874.

E-mail addresses: root@hamduni.ren.nic.in, baharchem@yahoo.com (B. Ahmed), almtanveer@yahoo.com (T. Alam), shahalamkhan@yahoo.com (S.A. Khan).

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2.2. Preparation of plant extracts

The seeds of *A. graveolens* and aerial parts of *C. oblongifolius* (8.0 kg) of each were dried and crushed to powder and then successively extracted to exhaustation with petroleum ether (60–80 °C), acetone and methanol using cold percolation method. The different extracts thus obtained were dried under reduced pressure to get the crude extract (400 g), (500 g) and (100 g) of *A. graveolens* and *C. oblongifolius*, respectively.

2.3. Phytochemical screening

On preliminary screening of *A. graveolens* and *C. oblongifolius*, the extracts showed the positive Shinoda test for flavonoids, Molisch test for glycosides, a positive Liebermann–Burchard reaction for steroid (Liebermann, 1885) and a positive Noller test for triterpenoids (Noller et al., 1942), which were confirmed by thin layer chromatography with the solvent system benzene–ethylacetate (1:1) over silica gel G (Stahl, 1969). Further separation of the specific phytochemical is in progress in our lab.

2.4. Experimental animals

The study was carried out on female Wistar Albino rats (100–150 g). The rats were bred in colony in the Central Animal House of Jamia Hamdard. They were fed with a standard pellet diet (Gold Mohar, Lipton India Ltd., Calcutta) and water ad libitum. Before their use in the experiment, the rats were kept in standard environmental conditions, (temperature 25–28 °C and 12 h light/dark cycle). Six groups of five animals each were used for each plant. Group I served as control, group II received the hepatotoxin (CCl_4), group III received the standard drug silymarin 10 mg/kg (p.o.) b/w and hepatotoxin. The remaining groups received the different extracts of the seeds of *A. graveolens* 250-mg/kg p.o. and aerial parts of *C. oblongifolius* 200-mg/kg p.o. and the hepatotoxin.

2.5. CCl_4-induced hepatotoxicity

CCl_4 was obtained from s.d. fine chem ltd., Bombay. Animals of the test groups were given the plants extracts in acacia gum (1%) in distilled water and standard drug silymarin, 24 h prior to the administration of CCl_4 given orally in dose of 1.5 ml/kg b/w. The animals of toxic group received vehicle, acacia gum (1%) in distilled water and CCl_4. The control group received vehicle alone. The biochemical parameters were determined 24 h after the CCl_4 challenge or administration.

2.6. Assessment of liver function

Rats of all groups were anaesthetized by diethyl ether, 24 h after the administration of hepatotoxin CCl_4. The blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30 °C for 15 min and analyzed for various biochemical parameters: serum transaminases viz. glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (SALP), total protein (TP) and albumin (TA) according to the reported methods (Reitman and Frankel, 1957; Kind and King, 1954; Wooton, 1964).

2.7. Statistical analysis

Results of the biochemical estimations are reported as mean $\pm$ S.E.M. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA), Student's *t*-test was used for determining significance (Woolson, 1987).

3. Results and discussion

Administration of CCl_4 led to increase the serum enzymes level by 2–3-folds as compared to control group. Treatment of rats with different extracts of *A. graveolens* (seeds) at dose of 250-mg/kg and *C. oblongifolius* (aerial parts) at a dose of 200-mg/kg b.w. p.o. markedly prevented CCl_4 induced elevation of serum GOT, GPT, SALP and increased the level of TP and TA (Tables 1 and 2).

CCl_4 induces fatty liver and cell necrosis (Pencil et al., 1984) and plays a significant role in inducing triacylglyceral accumulation, depletion of GSH, increased lipid peroxidation, membrane damage, depression of protein synthesis and loss of enzymes activity (Recknagel et al., 1989). Being cytoplasmic in location (Sallie et al., 1962) the damage marker enzymes GOT, GPT and LDH are released in serum (Chenoweth and Hake, 1962). It has been shown that protective agents exert their action against CCl_4 induced liver injury by impairment of CCl_4-mediated lipid peroxidation, either through decreased production of free radical derivatives (Castro et al., 1974) or due to the antioxidant activity of the protective agent itself (Yasuda et al., 1980). The extracts of the seeds of *A. graveolens* and aerial parts of *C. oblongifolius* used in the study preserved the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided as compared to the enzyme levels in CCl_4 treated rats.

Furthermore, protective mechanism not specific to CCl_4 may be responsible for hepatoprotective activity of the methanolic extracts of the seeds of *A. graveolens* and aerial parts of *C. oblongifolius*.

4. Conclusion

The above observations have shown that the methanolic extracts of the seeds of *A. graveolens* and aerial part of *C. oblongifolius* showed maximum anti-hepatotoxic activity, which should be related to the methanol soluble active principles like flavone and diterpene (Aiyar and Seshadri, 1970), whereas, the other extracts of seeds of *A. graveolens* and aerial part of *C. oblongifolius* also showed a lower antihepatotoxic activity. The activity of the tested samples was comparable to that of standard drug silymarin.

The isolation and testing of constituents likely to be responsible for the hepatoprotective activity of *A. graveolens* and *C. oblongifolius* is under progress in our lab.

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Table 1
Effect of various extracts of *A. graveolens* seeds on serum enzymes, alkaline phosphatase total proteins and albumin in CCl_4 induced liver damage in rats

Treatment	SGPT	SGOT	SALP	TP	TA
Control	65.5 ± 0.0	76.66 ± 3.35	31.75 ± 1.18	7.01 ± 0.18	3.85 ± 0.27
CCl_4	131.5 ± 1.99	169.16 ± 4.66	61.0 ± 0.99	5.58 ± 0.09	2.75 ± 0.08
Silymarin	64.38 ± 1.05*	73.33 ± 3.35*	31.25 ± 1.25*	7.29 ± 0.13*	4.0 ± 0.27
Petroleum ether extract	65.44 ± 1.05 (100)*	68.83 ± 1.17 (108.5)*	34.72 ± 0.55 (90.0)*	7.61 ± 0.06 (142.8)*	4.62 ± 0.05 (163.6)
Acetone extract	68.88 ± 5.5 (94.8)**	79.33 ± 4.66 (97.2)*	38.61 ± 1.39 (76.7)*	6.38 ± 0.39 (57.1)	4.57 ± 0.05 (163.6)
Methanol extract	64.38 ± 1.05 (101.6)*	51.33 ± 9.33 (127.5)*	32.69 ± 0.63 (96.9)*	8.0 ± 0.05 (171.4)*	5.28 ± 0.05 (227.3)*

Values are mean ± S.E.M., $n = 5$ animals per group. Figures in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatotoxin. The % of protection is calculated as 100× (values of CCl_4 control − values of sample)/(values of CCl_4 control − values of control). SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; SALP, alkaline phosphatase; TP, total protein; TA, total albumin; p.o., per oral.

* $P < 0.05$,

** $P < 0.01$ vs. CCl_4. One way analysis and Student's t-test.

Table 2
Effect of various extracts of *C. oblongifolius* plant on serum enzymes, alkaline phosphatase total protein and albumin in CCl_4 induced liver damage in rats

Treatment	SGPT	SGOT	SALP	TP	TA
Control	65.5 ± 0.0	76.66 ± 3.35	31.75 ± 1.18	7.01 ± 0.18	3.85 ± 0.27
CCl_4	131.5 ± 1.99	169.16 ± 4.66	61.0 ± 0.99	5.58 ± 0.09	2.75 ± 0.08
Silymarin	64.38 ± 1.05*	73.33 ± 3.35*	31.25 ± 1.25*	7.29 ± 0.13*	4.0 ± 0.27
Petroleum ether extract	126.66 ± 3.33 (7.44)	106.17 ± 2.33 (68.09)*	54.75 ± 3.33 (21.36)	6.72 ± 0.08 (79.72)*	4.04 ± 0.09 (117.2)**
Acetone extract	114.44 ± 8.89 (26.24)	103.83 ± 2.33 (70.62)*	53.81 ± 3.81 (24.58)	6.5 ± 0.07 (64.33)**	4.26 ± 0.13 (137.2)***
Methanol extract	56.66 ± 6.67 (115.13)*	40.83 ± 1.17 (138.73)*	34.36 ± 1.27 (91.09)*	10.43 ± 0.57 (339.16)**	4.89 ± 0.15 (194.5)

Values are mean ± S.E.M., $n = 5$ animals per group. Figures in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatotoxin. The % of protection is calculated as 100× (values of CCl_4 control − values of sample)/(values of CCl_4 control − values of control). SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; SALP, alkaline phosphatase; TP, total protein; TA, total albumin; p.o., per oral.

* $P < 0.05$,

** $P < 0.01$,

*** $P < 0.001$ vs. CCl_4. One way analysis and Student's t-test.

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