PHYTOTHERAPY RESEARCH
Phytother. Res. **16**, 256–260 (2002)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/ptr.854

Protective Effect of *Acorus calamus* Against Acrylamide Induced Neurotoxicity

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Exposure of rats to acrylamide (ACR) caused hind limb paralysis in 58% of the animals on day 10 and decreased behavioural parameters, namely distance travelled, ambulatory time, stereotypic time and basal stereotypic movements compared with the control group. These rats also had a decrease in the reduced glutathione (GSH) content and glutathione-S-transferase (GST) activity in the corpus striatum and an increase in striatal dopamine receptors, as evident by an increase in the binding of ^{3}H-spiperone to striatal membranes. Treatment with the ethanol:water (1:1) extract of the rhizomes of *Acorus calamus* (AC-002) increased the GSH content and GST activity in the corpus striatum while insignificant changes were observed in other parameters. Rats treated with ACR and AC-002 in combination had a lower incidence of paralysis (18%) compared with those treated with ACR alone on day 10 of the experiment. The rats also showed a partial recovery in other behavioural parameters. The levels of GSH content and GST activity increased in the corpus striatum, while the dopamine receptors decreased compared with the ACR treated rats. The results suggest that the neurobehavioural changes produced by ACR may be prevented following treatment with *Acorus calamus* rhizomes. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: acrylamide; *Acorus calamus*; reduced glutathione; glutathione-S-transferase; dopamine receptors.

INTRODUCTION

Acrylamide, a monomer, is a highly reactive molecule and has extensive applications in the production of polymers and co-polymers (Flock and Raush, 1973; Sussman and Wang, 1973; EHC-49, 1985). The monomer is a potent neurotoxic agent and has been reported to affect both central and peripheral nervous systems (Spencer and Schaumburg, 1987; LeQuesne, 1980; Agarwal *et al.*, 1981; Tilson, 1981; Ko *et al.*, 1999). Studies in experimental animals have revealed the primary role of glutathione and the involvement of dopamine receptors in acrylamide toxicity (Dixit *et al.*, 1980, 1981; Srivastava *et al.*, 1986). Human exposure to the monomer has been reported in both occupational and non-occupational conditions through inhalation, ingestion or dermal contact (EHC-49, 1985). *In vitro* and *in vivo* studies have shown that acrylamide binds to neurofilaments and inhibits fast axonal transport (Carrington *et al.*, 1991; Sickles *et al.*, 1996). The monomer has also been reported to inhibit the function of kinesin which catalyses the transport process (Sickles *et al.*, 1996). Exposure of humans to monomeric acrylamide is likely during experimental work because of its extensive use in laboratories (Costa *et al.*, 1992).

Several pharmacological agents and plant extracts have been found to be effective in preventing the neurotoxic effects of certain environmental chemicals although the mechanism is not clearly understood. One such plant could be *Acorus calamus* Linn (*Araceae*) which is widely found in Asia, Europe and North America. The rhizome are used extensively in the traditional Indian system of medicine for the treatment of epilepsy, hysteria, insomnia, neurosis etc., either as a single drug or as a component of certain drug preparations (Vohora *et al.*, 1990; Martis *et al.*, 1991). Two active principles namely α-asarone and *β*-asarone have been isolated, apart from the essential oil (Schmidt *et al.*, 1993). Alcohol and aqueous extracts of the root have been reported to be pharmacologically active. They increase the latency of seizures and reduce the mortality. The present study has been aimed at investigating the neuroprotective potential of the ethanol:water extract of rhizomes of *A. calamus*, named AC-002, on selected biochemical and behavioural parameters against acrylamide toxicity.

MATERIALS AND METHODS

The ethanol:water (1:1) extract of the rhizome of *A. calamus*, named AC-002, was prepared at the Regional Research Laboratory, Jammu, India.

Male albino rats of the Wistar strain, obtained from the Industrial Toxicology Research Centre animal breeding colony, weighing 100–110 g were used throughout the study. The animals were housed in polypropylene cages under standard hygiene conditions and were fed rat pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. The animals were divided into four groups and treatment was given as follows. Group I (control), normal saline;

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 Accepted 16 January 2001

Table 1. Effect of acrylamide on hind limb paralysis and protection by AC-002 in rats

Group	Day 6	Day 10
ACR (50 mg/kg)	5/12 (41%)	7/12 (58%)
ACR (50 mg/kg)+AC-002 (25 mg/kg)	0/12	2/11 (18%)

Figures indicate the number of animals paralysed of the total number of animals in each group.

group II (ACR, acrylamide, 50 mg/kg i.p. for 10 days; group III (AC-002), *Acorus calamus* extract, 25 mg/kg p.o. for 10 days; and group IV (AC-002 + ACR) a combination of AC-002 p.o. and ACR i.p. for 10 days.

The treatment with *Acorus calamus* extract (AC-002) was given simultaneously in the acrylamide treated group.

Behavioural studies. The change in sensory and motor behaviour of each rat was observed every day in each group. Further, the effects of acrylamide on various parameters of motor activity were studied using Optovarimex (Columbus Instruments, USA), 10 days after the treatment.

Biochemical studies. Rats in each group were killed 24 h after the last dose of treatment. The brain was immediately taken out and dissected to isolate the corpus striatum following the method of Glowinski and Iversen (1966) and processed for biochemical assays.

Estimation of reduced glutathione levels. The levels of reduced glutathione (GSH) in the corpus striatum were measured spectrophotometrically using 5,5′ -dithiobis (2-nitrobenzoic acid) as the colour reagent following the method of Hasan and Haider (1989) and the intensity of the yellow colour developed was read at 412 nm.

Assay of glutathione-S-transferase activity. The striatal tissue was homogenized in phosphate buffer (4 vol, 0.1M, pH 7.4) containing 0.15 M KCl and centrifuged at 14000 × **g** for 20 min at 4 °C. The supernatant, post mitochondrial fraction, was separated and the activity of glutathione-S-transferase was estimated by the method of Habig *et al.* (1974) using 1-chloro-2,4, di-nitrobenzene (CDNB) as the substrate.

Dopamine receptor assay. Assay of dopamine receptors in the corpus striatum was carried out by the method of Khanna *et al.* (1994). Briefly, crude synaptic membranes were prepared by homogenizing the tissue in 19 volumes of 0.32 M sucrose followed by centrifugation (50000×**g**, 10 min, 4 °C). Endogenous amine from the pellet was removed by washing with deionized water and centrifuging at the same speed for an additional 10 min. Finally, the pellet was suspended in 40 mM Tris-HCl, pH 7.4 and stored at −20 °C.

Binding incubations in a final volume of 1.0 mL were carried out in triplicate for 15 min at 37 °C. The incubation mixture contained 40 mM Tris-HCI, pH 7.4, together with labelled [1-phenyl-4-^{3}H-spiperone (18.5 Ci/mmol, 1×10^{-9} M) and unlabelled displacer (haloperidol, 1×10^{-6} M). The amount of tissue per tube corresponded to 300–400 μg membrane protein. At the end of incubation, the contents were rapidly filtered on glass fibre discs (Whatman GF/C, 25 μM pore size) and washed twice with 5 mL chilled buffer. The filters were dried and counted in 5 mL scintillation mixture (PPO, POPOP, methanol, dioxan, toluene and naphthalene). The radioactivity was measured using a LKB Rack β-scintillation counter at an efficiency of 30–40%. Basic binding characteristics including delineation of saturability, specificity, reversibility and regional distribution were established prior to the experiment.

Protein estimation. Protein content was measured following the method of Lowry *et al.* (1951) using bovine serum albumin as a reference standard.

Statistical analysis. Data were analysed by Student's *t*-test and $p < 0.05$ was considered significant.

RESULTS

Effect of acrylamide on motor behaviour including hind limb paralysis in rats and protective effect of *A. calamus*

Hind limb paralysis was observed in rats exposed to acrylamide on day 6 (41%). In contrast, no animal was paralysed in the group of rats treated with a combination of acrylamide and AC-002 on day 6. 58% rats in the ACR treated group were found paralysed on day 10 while in the rats treated with ACR and AC-002 in combination, the incidence of paralysis was 18% (Table 1). The rats treated with ACR showed a significant decrease in the distance travelled, ambulatory time, stereotypic time and basal stereotypic movements and an increase in the resting time, 10 days after exposure, in comparison with

Table 2. Effect on motor behaviour in rats following exposure to acrylamide and AC-002 for 10 days

Group	Distance Travelled (cm)	Resting Time (s)	Ambulatory Time (s)	Stereotypic Time (s)	Basal Stereotypic Movement
Control	691 ±108	73 ± 7.98	32 ± 4.35	73 ± 3.76	274 ± 23.36
ACR (50 mg/kg)	275 ± 100[a]	124 ± 12.69[a]	13 ± 4.41[a]	41 ± 8.6[a]	140 ± 31.8[a]
AC-002 (25 mg/kg)	814 ± 185	66 ± 9.44	36 ± 7.06	76 ± 3.74	282 ± 23.15
AC-002 (25 mg/kg) + ACR 50 mg/kg)	574 ± 179	92 ± 21.46	26 ± 7.85	61 ± 14.07	213 ± 55

Values are mean ±SE of five observations.
[a] Statistically significant from controls ($p < 0.05$).

Table 3. Effect of acrylamide and AC-002 exposure on reduced GSH levels and GST activity in corpus striatum

Group	GSH (μmol/g tissue)	GST (nmol conjugate formed/min/mg protein)
Control	0.371 ± 0.013	215 + 3.5
ACR (50 mg/kg)	0.265 ± 0.013[a]	148 + 5.5[a]
AC-002 (25 mg/kg)	0.690 ± 0.02[a]	183 + 8.4
AC-002 (25 mg/kg) + ACR 50 (mg/kg)	0.680 ± 0.12[b]	188 + 2.3[b]

Values are mean ± SE of four animal in each group;
[a] Compared with control rats $P < 0.05$;
[b] compared with ACR treated rats $p < 0.05$.

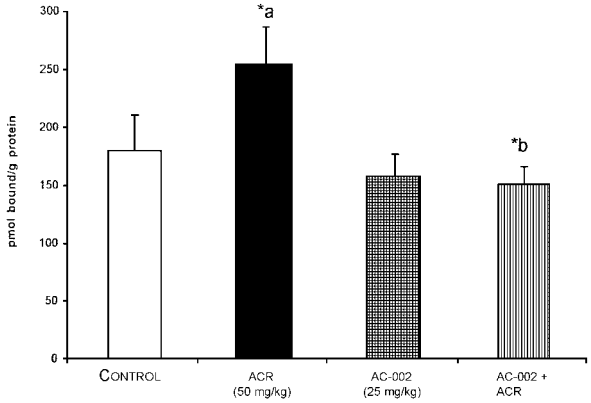

Figure 1. Effect of acrylamide and AC-002 exposure on striatal dopamine receptor. Values are mean ± SE of four animals in each group. *[a] Compared with control group. *[b] Compared with acrylamide treated group. *p, <0.05.

those treated with normal saline (Table 2). A marginal increase in the distance travelled was observed while there was no significant change in other parameters following treatment with AC-002 in comparison with saline treated controls. Interestingly, the rats treated with a combination of acrylamide and AC-002 showed protection in the behavioural parameters studied namely distance travelled, resting time, ambulatory time, stereotypic time and basal stereotypic movements compared with the rats treated with acrylamide alone.

Effect on reduced GSH levels. Exposure of rats to acrylamide caused a significant decrease in the reduced GSH levels in the corpus striatum (28%). However, rats treated with AC-002 alone exhibited a significant increase (85%) in the GSH level in the striatum compared with the saline treated controls (Table 3). Interestingly, treatment with acrylamide and AC-002 in combination caused a significant increase in the GSH levels in the corpus striatum compared with the acrylamide treated rats.

Effect on GST activity. A significant decrease in the GST activity (31%) was observed in the corpus striatum of rats following exposure to acrylamide while no significant effect was observed in the animals exposed to the plant extract. Rats treated with acrylamide and AC-002, in combination, exhibited an increased activity of GST compared with the rats treated with acrylamide alone, although the activity of the enzyme was a little less than that of the control rats (Table 3).

Effect on striatal dopamine receptors. Exposure of rats to acrylamide for 10 days increased the striatal dopamine receptors as evidenced by an increase in the binding of ^{3}H-spiperone to striatal membranes, compared with the saline treated controls (Fig. 1). Treatment with acrylamide and AC-002 in combination decreased the binding in the striatal membranes compared with the acrylamide treated rats.

DISCUSSION

Several factors, the depletion of cellular glutathione, inhibition of the glutathione-S-transferase (GST) activity, mitochondrial dysfunction and altered protein metabolism, have been reported to modulate acrylamide neurotoxicity (Edwards, 1975; Dixit *et al.*, 1981; Das *et al.*, 1982; Spencer *et al.*, 1979; Sabri and Spencer, 1980; Husain *et al.*,1986). Exposure to the monomer has also been found to alter the neurotransmitter levels, their receptors and associated enzymes involved in the metabolism of neurotransmitters and cause neurotoxicity (Agarwal *et al.*, 1981; Ali *et al.*, 1983; Husain *et al.*, 1987). Enhanced striatal dopamine receptors and decreased GST activity were reported to be the primary changes in acrylamide neurotoxicity in rats since no toxic effect on these parameters was observed following treatment with N,N′-bis acrylamide, a non-neurotoxic analogue (Srivastava *et al.*, 1986). A decrease in the reduced GSH level and GST activity and increased striatal dopamine receptors observed in the present study is consistent with earlier reports and suggests a vulnerability of striatal dopamine receptors and GST activity to acrylamide.

Various chemical and pharmacological analogues including those acting on enzymes as a substrate such as sodium pyruvate or as a cofactor (pyridoxine) have been reported to attenuate the neurobehavioural toxic effects of acrylamide (Loeb and Anderson, 1981; Dairman *et al.*, 1981). In the present study, concurrent administration of AC-002 extract with acrylamide could protect the rats from hind limb paralysis which was significantly reduced compared with those treated with acrylamide alone. Interestingly, these rats also showed significant recovery in the reduced GSH levels, GST activity and dopamine receptors. Treatment of rats with the plant extract significantly increased the GSH levels although it had no effect on the GST activity and dopamine receptors. The reason for such an effect is still not clear. Possibly, the increased GSH levels due to AC-002 could be responsible for the protective effect in acrylamide exposed rats. Since acrylamide easily conjugates with agents containing free -SH groups, treatment of animals with methionine, a sulphur containing amino acid, has been reported to inhibit significantly acrylamide neurotoxicity (Hashimoto and Ando, 1973).

Studies on the alcohol extract of *Acorus calamus* have shown the presence of β-asarone, an active ingredient (Vohora *et al.*, 1990). Both the roots and rhizomes of the

plant have been used in the Ayurvedic system for the treatment of epilepsy, neurosis, insomnia and other diseases (Vohora *et al.*, 1990; Martis *et al.*, 1991). In a recent study on diabetic rats, the neurotoxic effects of acrylamide were found to be decreased, as judged by functional parameters such as hind limb function, landing foot splay etc. compared with non-diabetic animals (Deeb *et al.*, 2000). Husain *et al.* (1989) reported that pre-treatment of rats with six mycelial fraction acetone (6-MFA), an interferon inducer of fungal origin, could significantly inhibit acrylamide neurotoxicity. In another study, these authors reported that post-treatment with 6-MFA in the acrylamide treated rats could also reverse the toxic effects of the monomer (Husain *et al.*, 1991). Although the precise mechanism for such an effect could not be established, the interferon inducing capability of 6-MFA was suggested in the prevention of neurotoxic effects of acrylamide.

The present study indicates that a decrease in GSH levels and GST activity and an increase in dopamine receptors in the corpus striatum by acrylamide can be prevented by treatment with AC-002. However, the exact mechanism is yet to be worked out.

Acknowledgements

The authors acknowledge financial support by the Council of Scientific and Industrial Research, New Delhi which made this study possible. They also wish to thank the Director, Industrial Toxicology Research Centre, Lucknow for his interest in the study and for providing all the facilities.

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