# Hepatoprotective Activity of Quercetin against Acrylonitrile-Induced Hepatotoxicity in Rats

*Osama M. Abo-Salem*[1], *Mohamed F. Abd-Ellah*[1], *and Mabrouk M. Ghonaim*[2]
[1]Department of Pharmacology & Toxicology, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt
[2]Department of Microbiology and Immunology, Faculty of Medicine, Menoufiya University, Shebin El-Kom, Egypt

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**ABSTRACT: Acrylonitrile is a potent hepatotoxic, mutagen, and carcinogen. A role for free radical-mediated lipid peroxidation in the toxicity of acrylonitrile has been suggested. The present study was designed to assess the hepatoprotective effect of quercetin against acrylonitrile-induced hepatotoxicity in rats. Liver damage was induced by oral administration of acrylonitrile (50 mg/kg/day/5 weeks). Acrylonitrile produced a significant elevation of malondialdehyde (138.9%) with a marked decrease in reduced glutathione (72.4%), and enzymatic antioxidants; superoxide dismutase (81%), and glutathione peroxidase (53.2%) in the liver. Serum aspartate aminotransferase, alanine aminotransferases, direct bilirubin, and total bilirubin showed a significant increase in acrylonitrile alone treated rats (115.5%, 110.8%, 1006.8%, and 1000.8%, respectively). Pretreatment with quercetin (70 mg/kg/day/6 weeks) and its coadministration with acrylonitrile prevented acrylonitrile-induced alterations in hepatic lipid peroxides and enzymatic antioxidants as well as serum aminotransferases and bilirubin. Histopathological findings supported the biochemical results. We suggest that querectin possess hepatoprotective effect against acrylonitrile-induced hepatotoxicity through its antioxidant activity.** © 2011 Wiley Periodicals, Inc. J Biochem Mol Toxicol 25:386–392, 2011; View this article online at wileyonlinelibrary.com. DOI 10:1002/jbt.20406

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## INTRODUCTION

Acrylonitrile (ACN) is widely used in the production of many industrial products such as synthetic fibers, nitrile rubbers, resins, plastics, and pesticide fumigants for grains. It is also used as an intermediate in the synthesis of antioxidants, surface coatings, adhesives, and dyes [1]. In clinical practice, ACN is used in the synthesis of high permeable dialysis tubings [2], and artificial membranes to encapsulate Langerhan's islet implants [3]. Moreover, ACN has been found in water, occupational environments, food, and cigarette smoke [3].

ACN is metabolized through different metabolic pathways: (a) enzymatic (glutathione-*S*-transferase "GST") [4] and nonenzymatic conjugation with reduced glutathione (GSH) and protein sulfhydryl groups (the major route) [5] and (b) oxidation by the cytochrome P-450 to yield 2-cyanoethylene oxide and cyanide (CN) (the critical step in ACN toxicity) [6].

Chronic exposure of rats to ACN has been reported to produce a dose-related increase in glial cell tumors (astrocytomas) [7]. Moreover, previous studies have shown that ACN exposure was associated with induction of oxidative stress (OS) and reduction in tissue content of cysteine [8], lysine, methionine, and GSH as well as disturbance of pyruvate metabolism [9]. Furthermore, increased lipid peroxidation, oxidative damage (OD) of DNA, and deficiency of antioxidant protection in rat brain were reported following ACN chronic exposure [10]. Acute ACN toxicity in rats included tissue damage of important organs (brain, liver, lung, and kidney) [11], gastric and duodenal ulceration [5], and adrenocorticolysis [11].

Flavonoids are characterized by many pharmacodynamic actions as hypolipidemic, cardiotonic, hypotensive, antiarrhythmic, spasmolytic, sedative, diuretic, hypothermic, and antibacterial effects [12]. Quercetin, a flavonoid antioxidant, is one of the flavonols that are commonly found in vegetables [13].

*Correspondence to*: Osama M. Abo-Salem; e-mail: oabosalem@yahoo.com
Present address of Osama M. Abo-Salem and Mabrouk M. Ghonaim: Departmentof Medical Laboratories, College of Applied Medical Sciences, Taif University, Saudi Arabia.

Previous studies showed that quercetin protected the liver and lung against toxicity induced by ammonium fluoride [14] and beta-cell damage induced by streptozotocin in rat pancreas [15]. The liver is the main site of oxidative and detoxifying processes. In many diseases, elevated biomarkers of OS were detected in the liver at early stages [16].

This study was performed to investigate a potential protective effect of quercetin treatment on liver OS in ACN-induced hepatic toxicity in rats.

## MATERIALS AND METHODS

### Chemicals

All chemicals and kits were purchased from Sigma-Aldrich Company (St. Louis, MO) and Biodiagnostics Company (Cairo, Egypt).

### Animals

Adult male albino rats (130–150 g) of Wistar strain were used and purchased from the National Institute of Cancer, Cairo, Egypt. They were housed in clean cages, fed with commercial rat chow and water ad libitum under standard laboratory conditions. All animal experiments were conducted according to the regulations of the Committee on Bioethics for Animal Experiments of Al-Azhar University.

### Experimental Design

Fifty rats were used and divided into five groups ($n = 10$ in each group).

- Group I: served as a control group (*control*) and received distilled water, p.o., daily for 6 weeks.
- Group II: served as vehicle treated group (*vehicle*) and received ethanol in water (1:9), p.o., daily for 6 weeks.
- Group III: Quercetin alone treated group (*Q*) and received daily quercetin for 6 weeks in a dose of 70 mg/kg bwt, p.o., dissolved in the vehicle [13,17].
- Group IV: served as ACN alone treated group (*ACN*) and received an aqueous solution of ACN (50 mg/kg bwt, p.o, half of the LD50), once daily for 5 weeks [18].
- Group V: served as quercetin + ACN treated group (*Q + ACN*) and received quercetin (70 mg/kg bwt) 1 week before starting ACN treatment as well as concomitantly with ACN (50 mg/kg bwt) once daily for 5 weeks. Animals were treated with ACN at 2 h after quercetin administration.

The dose of quercetin that was previously reported to induce maximal beneficial effects in different liver diseases was used [13]. All treatments were given in a volume of 10 mL/kg bwt.

### Assessment of Serum and Hepatic Biochemical Markers

After 24 h of the last dose, the animals were sacrificed under ether anesthesia and blood was collected. The blood sample of each animal was collected separately by carotid artery into sterilized dry centrifuge tubes and allowed to coagulate for 30 min. Serum was separated by centrifugation at 3000 rpm for 15 min. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin were estimated using an autoanalyzer [19,20].

The livers were removed immediately, washed with ice-cold saline, and a 10% homogenate was prepared in phosphate buffered saline (pH 7.0). The homogenate was centrifuged at 3000 rpm for 15 min at 4°C, and the supernatant was used for estimation of reduced GSH [21], malondialdehyde (MDA) [22], superoxide dismutase (SOD) [23], glutathione peroxidase (GSHpx) [24], and protein [25]. Livers of two rats were preserved in 10% formaldehyde solution for the histological study.

### Histopathological Examination

Liver sections were cut at 5-μm thickness, using a rotary microtome and stained with haematoxylin and eosin [26]. Sections were examined and photographed under a light microscope.

### Statistical Analysis

The results were expressed as mean ± SEM and statistically analyzed using one-way analysis of variance (ANOVA) followed by Student–Tukey–Kramer test. $P$ values $\leq 0.05$ were considered significant. The percentage of change was calculated as follows: (treated – control)/(control) × 100.

## RESULTS

### Liver Function Tests

The effects of quercetin on ACN-induced liver damage in rats with reference to biochemical changes

**TABLE 1.** Effect of Acrylonitrile and/or Quercetin on Serum Levels of Aminotransferases and Bilirubin

| | Studied Liver Function Tests | | | |
|---|---|---|---|---|
| Treatment | AST (IU/L) | ALT (IU/L) | Total Bilirubin (mg/dL) | Direct Bilirubin (mg/dL) |
| Control | $42.63 \pm 2.49$ | $58.38 \pm 4.77$ | $0.117 \pm 0.006$ | $0.029 \pm 0.003$ |
| Vehicle | $41.50 \pm 3.26$ | $63.25 \pm 6.22$ | $0.112 \pm 0.008$ | $0.030 \pm 0.002$ |
| Q | $45.13 \pm 3.67$ | $63.88 \pm 4.86$ | $0.111 \pm 0.008$ | $0.034 \pm 0.003$ |
| ACN | $91.88 \pm 4.20^{a,b,c}$ | $123.1 \pm 5.54^{a,b,c}$ | $1.288 \pm 0.084^{a,b,c}$ | $0.321 \pm 0.023^{a,b,c}$ |
| ACN + Q | $52.50 \pm 4.28^{d}$ | $63.13 \pm 4.98^{d}$ | $0.140 \pm 0.005^{d}$ | $0.038 \pm 0.002^{d}$ |

Data are expressed as mean ± SEM of eight rats for each group.
Q: quercetin; ACN: acrylonitrile.
[a] Significantly different from control group.
[b] Significantly different from vehicle treated group.
[c] Significantly different from rats treated with Q alone.
[d] Significantly different from group treated with ACN alone using one-way ANOVA with Tukey–Kramer test at $P \leq 0.05$.

in serum are presented in Table 1. At the end of the 5 weeks treatment, ACN alone treated animals showed a significant increase in the serum levels of AST (115.5%), ALT (110.9%), direct bilirubin (1006.8%), and total bilirubin (1000.8%) as compared to the control group. On the other hand, treatment of rats with quercetin (1 week before and concomitantly with ACN) prevented the ACN-induced increase in serum AST, ALT, and bilirubin as compared to the ACN alone treated group.

## Lipid Peroxides and Antioxidant Levels in the Liver

The hepatic MDA content was significantly increased by 138.9% in ACN-treated rats. On the other hand, there was a marked reduction in the hepatic content of GSH, SOD, and GSHpx amounting to 72.4%, 81%, and 53.2%, respectively, in ACN alone treated animals. Pretreatment with quercetin and itscoadministration with ACN ameliorated the alterations in the hepatic MDA, GSH, SOD, and GSHpx (Table 2).

## Histopathological Findings

Figure 1 shows that there were significant changes in microscopic photographs of the liver in ACN alone treated animals as compared to the normal control group. Normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein were observed in the normal control, vehicle, and quercetin alone treated groups (Figures 1A, 1B, and 1C). On the other hand, the ACN alone treated group exhibited histopathological changes, such as portal inflammation and tissue disorganization. The hepatocytes adjoining the portal tract show ballooning degeneration and droplet fatty change with infiltrating lymphocytes (Figure 1D). Pretreatment of rats with quercetin prevented the histopathological changes associated with hepatic toxicity induced by ACN. However, mild inflammatory cell infiltration, and fatty change are still observed (Figure 1E).

**TABLE 2.** Effect of Acrylonitrile and/or Quercetin on Liver Content of Oxidant and Antioxidant Parameters

| | Studied Hepatic Tissue Parameters | | | |
|---|---|---|---|---|
| Treatment | GSH (μg/g tissue) | MDA (nmol/mg protein) | GSHpx (nmol/mg protein/min) | SOD (U/mg protein) |
| Control | $16.54 \pm 0.53$ | $3.24 \pm 0.10$ | $1208.0 \pm 68.19$ | $13.95 \pm 1.05$ |
| Vehicle | $15.53 \pm 0.69$ | $3.33 \pm 0.27$ | $1205.0 \pm 77.93$ | $13.95 \pm 1.18$ |
| Q | $15.20 \pm 0.86$ | $3.57 \pm 0.22$ | $1231.7 \pm 95.67$ | $14.98 \pm 0.73$ |
| ACN | $4.57 \pm 0.27^{a,b,c}$ | $7.74 \pm 0.39^{a,b,c}$ | $565.3 \pm 44.10^{a,b,c}$ | $2.58 \pm 0.17^{a,b,c}$ |
| ACN + Q | $14.00 \pm 1.25^{d}$ | $3.97 \pm 0.34^{d}$ | $1239.2 \pm 61.45^{d}$ | $16.53 \pm 1.10^{d}$ |

Data are expressed as mean ± SEM of eight rats for each group.
Q: quercetin; ACN: acrylonitrile.
[a] Significantly different from control group.
[b] Significantly different from vehicle treated group.
[c] Significantly different from rats treated with Q alone.
[d] Significantly different from group treated with ACN alone using one-way ANOVA with Tukey–Kramer test at $P \leq 0.05$.

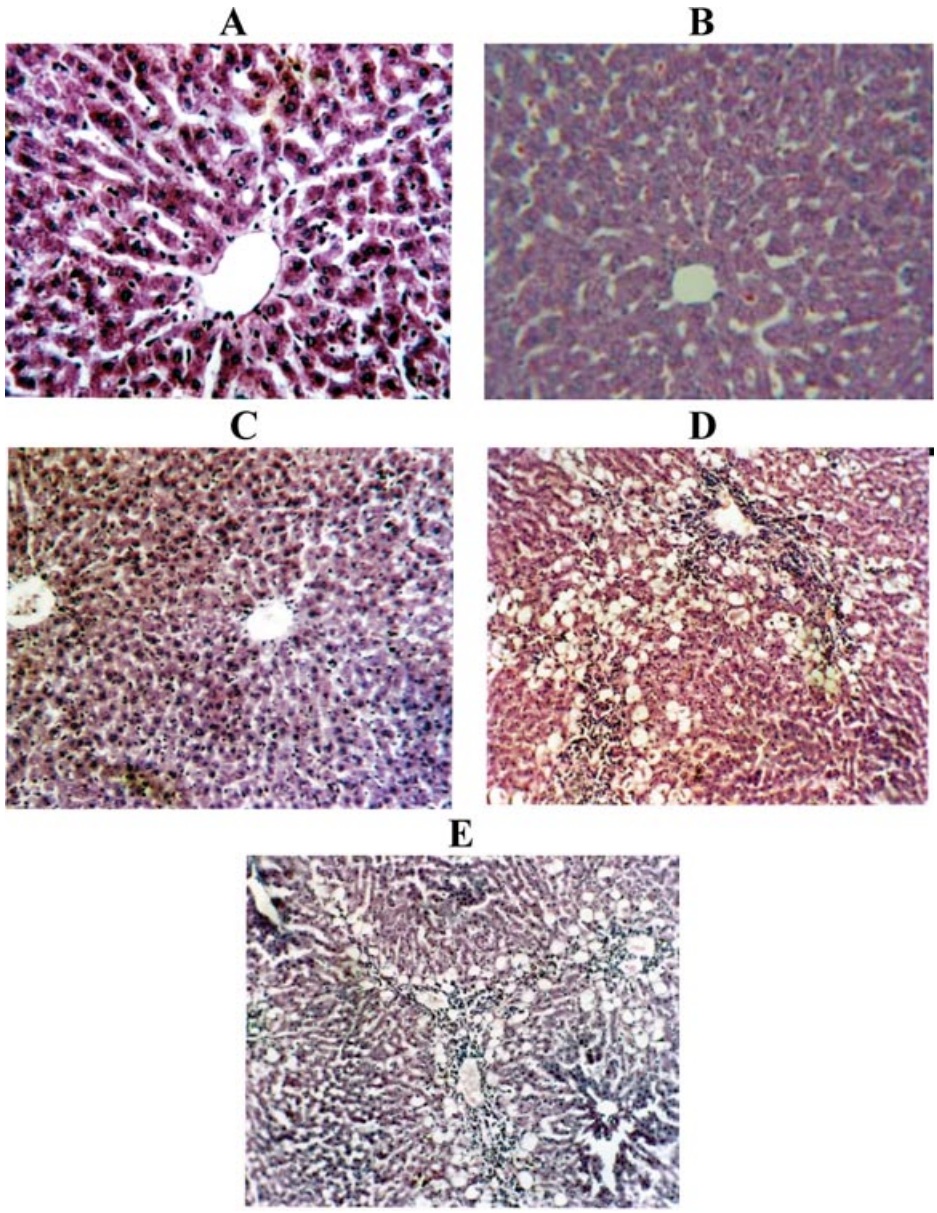


**FIGURE 1.** Histopathological changes of rat liver treated with ACN and/or quercetin (H&E stain; × 300). (A): Normal rat liver, (B): vehicle, (C): quercetin alone, (D): positive control (ACN), (E): quercetin + ACN. Sections (A), (B), and (C) show a normal structure of the hepatic cells. Section (D) shows hepatocyte degeneration, large droplet fatty change and infiltrating lymphocytes. Treatment of rats with quercetin + ACN shows that mild inflammatory cell infiltration and fatty change are still observed (section (E)).

## DISCUSSION

Several studies have indicated the role of OS in ACN toxicity [27,28]. The major pathway of ACN elimination is its conjugation with GSH to form mercapturic acid through GST and production of CN via cytochrome P450 [4].

The present study showed a decrease in both enzymatic (SOD and GSHpx) and nonenzymatic (GSH) antioxidants as well as an increased level of lipid peroxidation (represented by MDA) in the ACN alone treated rats. Similar findings were reported by Mahalakshmi et al. [28]. GSHpx is responsible for the removal of ROS, such as peroxides. ACN may diminish the activities of GSHpx enzyme by inhibition of enzyme synthesis or inactivation by conjugation [28,29]. Free radical scavengers such as GSH, vitamin E, silymarin, hesperidine, taurine, and quercetin exist in their interconvertible forms and participate in the detoxification of reactive oxygen species (ROS) [18,28]. By depleting

GSH, ACN may decrease the antioxidant levels in cells leading to an overall increase in intracellular ROS and OD. CN has been shown to induce OS in mice brain by inhibiting mitochondrial respiratory chain, catalase, and GSHpx [30]. GSH participates in enzymatic reduction of membrane hydroperoxy-phospholipids and prevents the formation of secondary alkoxyl radicals when peroxides are homolyzed [31].

$$\text{La-OOH} + 2\text{GSH} \xrightarrow[\text{or GST-}\alpha]{\text{Se-Gpx}} \text{La-OH} + \text{GSSG} + \text{H}_2\text{O}$$

$$\text{PH-OOH} + 2\text{GSH} \xrightarrow[\text{and/or GST-}\alpha]{\text{PH-Gpx}} \text{PH-OH} + \text{GSSG} + \text{H}_2\text{O}$$

where PH is phospholipids, La is fatty acid, Seis selenium, Gpx is glutathione peroxidase, GSSG is oxidized glutathione [32].

Binding of ACN to GSH results in induction of OS and impaired regeneration of other antioxidants [33]. Studies with $^{14}$C ACN have shown that ACN covalently binds with sulfhydryl groups of protein [34], macromolecules, and nucleic acids [1]. This explains the reduction in the GSH content in cells and increases their susceptibility to OD.

Our results indicated that quercetin treatment significantly decreased ACN-induced lipid peroxidation, accompanied by the increased hepatic GSH content and enhanced activities of SOD and GSHpx. Quercetin attenuates lipid peroxidation either by scavenging and inactivating $H_2O_2$ and hydroxyl radicals or by binding with copper and iron [13,35–37]. Quercetin may also terminate lipid peroxidation by inducing enzymatic and nonenzymatic antioxidants. Our results are consistent with those of Wadsworth and Koop, [38] who demonstrated that quercetin improved hepatic levels of GSH, MDA, SOD, and GSHpx in diabetic rats, possibly by preventing cytotoxic effects of nitric oxide (NO) and ROS.

The present study revealed that ACN (50 mg/kg) produced significant elevations in ALT, AST, and bilirubin. These parameters have been reported to be sensitive indicators of liver injury [27]. Increased levels of aminotransferases showed that the integrity of hepatocytes was abnormal in ACN alone treated rats, resulting in the release of these enzymes into the systemic circulation [39]. Administration of quercetin prevented ACN-induced elevations of AST (50.9%), ALT (48.1%), and total (91.4%) and direct bilirubin (89.4%) as compared to ACN alone treated group. Kebieche et al. [40] reported that quercetin ameliorated the altered enzyme levels and protected rat liver against chemicals- or drugs-induced hepatotoxicity. Quercetin may inhibit lipid peroxidation by blocking xanthine oxidase, binding with iron and/or copper [41], scavenging hydroxyl, peroxy, and superoxide radicals [42], increasing vitamin C absorption [43], and inducing nitric oxide synthase inhibitor (decreasing NO and peronitrate generation) [44]. Histopathological investigations demonstrated that ACN-induced various degenerative changes in the hepatocytes, which confirmed the biochemical data. Treatment with quercetin obviously mitigated the histopathological changes induced by ACN.

## CONCLUSION

ACN-induced hepatic damage might be related to OS. Treatment with quercetin decreased the ACN-induced elevation in biochemical parameters and was effective in structural improvement of liver. Our results suggest that quercetin may have a potential therapeutic effect against ACN-induced liver injury.

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