Protective Effects of Curcumin Against Formaldehyde-induced Renal Toxicity in Rats

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ABSTRACT

Objective: *To explore the protective effects of curcumin against renal injury induced by formaldehyde in rats.*

Methods: *A total of 21 male Sprague–Dawley rats were included. The animals were divided into three groups. The control group received 10 ml/kg of physiological saline intragastrically and intraperitoneally on a daily basis. The formaldehyde group were given 10 ml/kg of physiological saline intragastrically plus 10 mg/kg of formaldehyde intraperitoneally. The formaldehyde + curcumin group received 10 mg/kg of intraperitoneal formaldehyde daily as well as 100 mg/kg of curcumin intragastrically. After the completion of 14 days, the kidneys were removed. Tissue microscopic examination was performed with haematoxylin–eosin and periodic acid–Schiff staining. Also, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and xanthine oxidase (XO) activities, and malondialdehyde (MDA) and nitric oxide (NO) levels were measured in tissue samples.*

Results: *Formaldehyde induced renal injury. The degenerative tissue changes in the formaldehyde + curcumin group seemed to regress, exhibiting similar characteristics to those of the controls. MDA, XO and NO were significantly higher in formaldehyde group than in controls, while a significant reduction occurred in SOD, CAT and GSH-Px activities in the formaldehyde group. Also, renal tissue MDA, XO and NO were significantly lower in the formaldehyde + curcumin group than in the formaldehyde group, while tissue SOD, CAT and GSH-Px activities were significantly higher.*

Conclusion: *Curcumin improved the formaldehyde-induced renal degeneration. Also, curcumin was found to prevent the reduction in SOD, CAT and GSH-Px activities, while preventing MDA, XO and NO levels, exhibiting a protective effect against the formaldehyde-induced oxidative renal injury.*

Keywords: Curcumin, formaldehyde, kidney, oxidative stress, renal toxicity.

INTRODUCTION

Formaldehyde, a colourless liquid with strong hydrophilic characteristics and odour, is commonly used in medicine and industry (1). After introduced into the organism, formaldehyde is converted into formic acid in the liver and erythrocytes by the catalytic activity of formaldehyde dehydrogenase, and is excreted without accumulation in the body through urine and faeces as well as in the form of carbon dioxide through respiration (2). In rats, systemic administration of formaldehyde has been shown to induce nephrotoxicity (3, 4).

Curcumin has a wide spectrum of biological and pharmacological effects including antioxidant, anti-cancerogen, antimutagenic, antidiabetic, antibacterial,

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antiviral, anti-inflammatory, and anti-nociceptive effects (5, 6). Curcumin inhibits the nitric oxide synthase activity. Due to its ability to bind to free radicals, it protects DNA from oxidative damage. Its antiapoptotic effects result from the antioxidant properties (7, 8). In rat study, 60% of an oral dose of curcumin was found to be absorbed, and the great majority of the remaining dose was detected in faeces and in the urine in the form of glucuronide and sulphate conjugates (9). Several studies have also reported protective effects of curcumin against renal injury in rats induced by a variety of different substances (10, 11).

This study was undertaken to examine the renoprotective effects of curcumin against formaldehyde-induced nephrotoxicity in rats.

MATERIALS AND METHODS

The experimental protocol used in this study was reviewed and approved by the Local Animal Ethics Committee of Namik Kemal University, Tekirdag, Turkey, in accordance to National Institutes of Health guidelines for the care and use of laboratory animals.

In this study, a total of 21 biologically and physiologically similar male Sprague-Dawley rats kept in standard conditions were divided into three groups:

Control group (n = 7): For 14 days, 10 ml/kg of physiological saline was administered intragastrically and intraperitoneally on a daily basis.

Formaldehyde group (n = 7): For 14 days, 10 ml/kg of physiological saline was administered intragastrically and 10 mg/kg of formaldehyde was administered intraperitoneally on a daily basis.

Formaldehyde + curcumin group (n = 7): For 14 days, 10 mg/kg of formaldehyde was administered intraperitoneally plus 100 mg/kg of curcumin was given intragastrically on a daily basis.

At the completion of the 14 days, the rats were sacrificed with ketamine–xylazine anaesthesia and the renal tissues were removed for histopathological and biochemical assessments.

Microscopic examination of kidney tissue specimens

The kidney tissue specimens were fixed in neutral formalin solution (10%). Tissue specimens were embedded in paraffin wax and sectioned (thickness, 5 mm). Paraffin sections were used for light microscopic examination. For light microscopic evaluation, paraffin sections were stained with haematoxylin–eosin and periodic acid–Schiff (PAS) and examined with an Olympus BX41 light microscope.

Biochemical tests

Xanthine oxidase (XO) and catalase (CAT) enzyme activities and malondialdehyde (MDA), and nitric oxide (NO) levels of the kidney tissues were determined spectrophotometrically in the biochemical examinations. Activities of tissue superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were determined by enzyme-linked immunosorbent assay.

Tissue homogenization and sample preparation

The wet tissues were set to 1 g, stored cold and divided into pieces with clean surgical scissors. A 2 ml Tris–HCl buffer was added to the tissue, which was previously put into a glass tube. The tissue in the glass tube was placed into an ice-filled container and homogenized at a speed of 16 000 rev/min. A buffer, 10 times the final volume of the tissue, was added. A second homogenization was carried out and finished in 3 minutes. Without increasing the homogenate temperature, it was transferred into Eppendorf tubes and coded. The wet tissue weight and added buffer amount were recorded. Malondialdehyde specifications were carried out with the obtained homogenates. Supernatant was obtained by centrifuging the homogenates at 3220 rpm/430 minutes + 6°C in a refrigerated centrifuge. Catalase and protein specifications of the separated supernatants were carried out. With a rate of 1/1 (v/v), supernatant was vortexed with chloroform/ethanol (3/5, (v/v) (28) and centrifuged in a glass tube at 3220 rpm/40 minutes + 4°C. Protein and SOD enzyme activity specification of the ethanol phase on the top was carried out.

Superoxide dismutase enzyme activity determination

Tissue SOD levels were determined using commercial kits (Eastbiopharm, CK-E30267, Hangzhou, China) and were expressed as ng/mg protein.

Catalase enzyme activity determination

Catalase activity was determined according to the method of Aebi (40). Results were expressed as kg/g.

Glutathione peroxidase enzyme activity determination

Tissue GSH-Px activities were measured using commercial kits (Eastbiopharm, CK-E90555, Hangzhou, China), and the results were expressed as ng/mg protein.

Determination of MDA levels

The tissue MDA levels were determined by the method of Draper and Hadley (12) based on the reaction of

MDA with thiobarbituric acid (TBA) at 95°C. Results were expressed as nmol/g protein.

Measurement of tissue XO activity

Xanthine oxidase activity was assayed spectrophotometrically at 293 nm and 37°C with xanthine as substrate (13). Results were expressed as U/g protein.

Measurement of tissue NO level

As NO measurement is very difficult in biologic specimens, sample nitrite and nitrate concentrations are used as an index of NO production. The method was based on the Griess reaction (14). Results were expressed as μmol/g protein.

Protein analysis

The protein levels of the samples were determined according to the recommended method of Lowry *et al* (15).

Statistical analysis

Statistical analysis was performed with 'PASW® Statistics 18 for Windows' (SPSS Inc., Chicago, IL, USA) software program. Results were expressed as mean ± standard deviation. The obtained '*p*' value of < 0.05 was considered to be statistically significant. We used one-sample Kolmogorov–Smirnov for the assessment of the normal distribution. 'One-way analysis of variance' test was used for the comparison of the differences between the groups. The groups were compared in pairs with 'Tukey honestly significant difference' which is one of the post hoc tests.

RESULTS

Light microscopic findings

In the control group, collecting tubules of kidney were a normal histological appearance (Fig. 1a). In the formaldehyde group, quite prominent dilatation of kidney collecting tubules was seen (Fig. 1b). In the formaldehyde + curcumin group, dilatation of kidney collecting tubules was less than that in the formaldehyde group (Fig. 1c). In the light microscopic evaluation of the kidney tissue specimens, glomeruli and tubules were observed as normal in the control group (Fig. 2a). In the formaldehyde group, quite prominent dilatation of the proximal and distal tubules and significant PAS positivity of basal membranes of the proximal and distal tubules were determined (Fig. 2b). In kidney section of rats treated with formaldehyde and curcumin, dilatation and
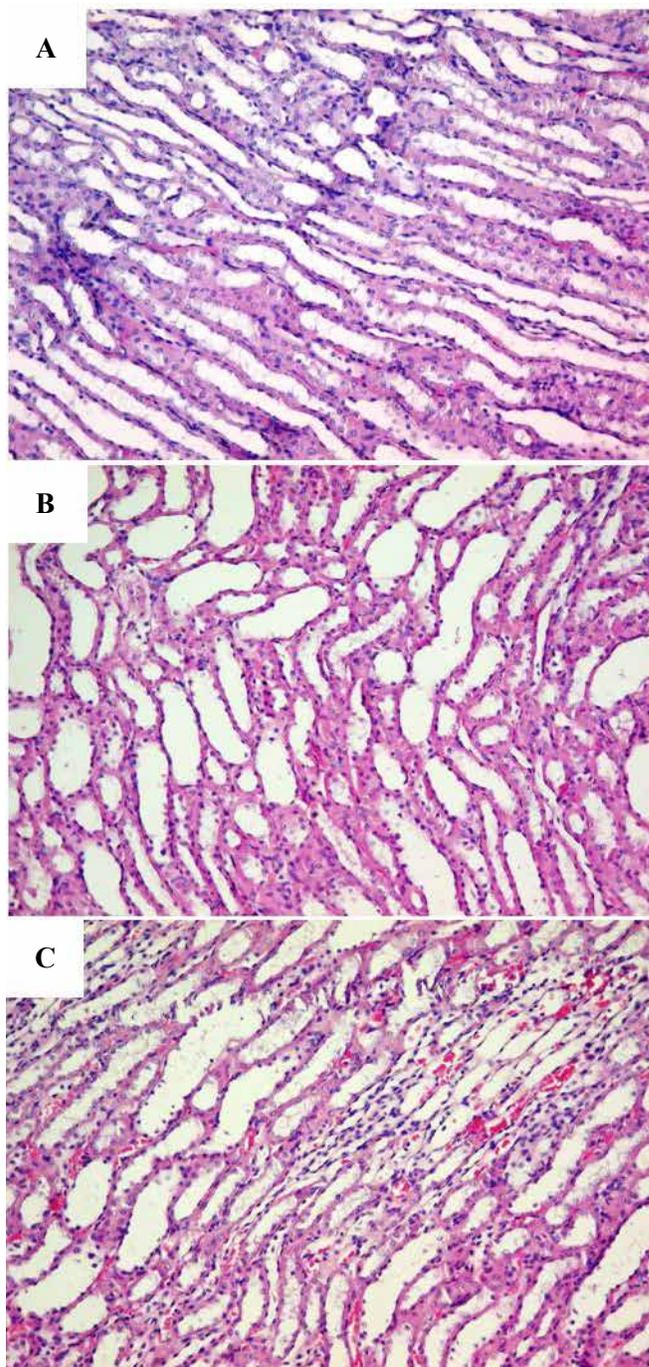

Figure 1.   Light microscopy of kidney tissue in different groups by Hematoxylin-Eosin stain (H&E): (A) Section of collecting tubules of kidney from control group showing a normal histological appearance. (B) Dilatation of kidney collecting tubules was seen in the kidney of rats treated with formaldehyde. (C) In formaldehyde+curcumin group, dilatation of kidney collecting tubules was less than in formaldehyde group. 200x magnification.
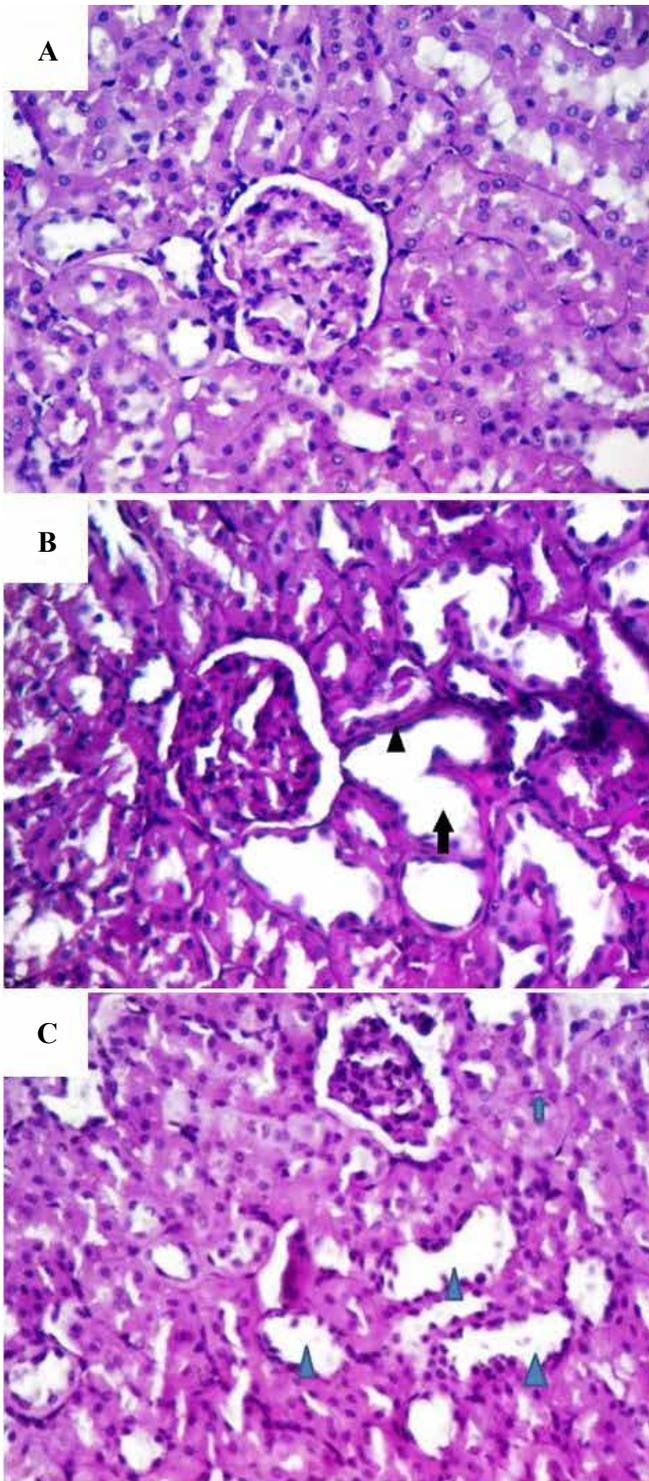
Figure 2. Light microscopy of kidney tissue in control, formaldehyde and formaldehyde+curcumin groups by Periodic Acid Schiff stain (PAS): (A) Kidney section from control group showing a normal histological appearance. (B) Dilatation of kidney tubules (arrow) and significantly PAS positivity of basal (arrow head) of tubules were seen in the kidney of rats treated with formaldehyde. (C) Section of kidney tubules of rats treated with formaldehyde and curcumin. Dilatation of kidney tubules (ahead arrow) and PAS positivity of basal of kidney tubules (ahead) were less than treated with formaldehyde. 400x magnification.

PAS positivity of the proximal and distal tubules were less than that in the formaldehyde group (Fig. 2c).

The results of biochemical analyses in renal tissues
The SOD, CAT, GSH-Px, XO activities and MDA and NO levels in renal tissues from study groups are shown in Tables 1 and 2. As compared to control rats, a significant higher MDA, which is a marker of lipid peroxidation, was found in rats that received intraperitoneal formaldehyde. On the other hand, rats in the curcumin + formaldehyde group had significantly lower MDA level than in rats that received formaldehyde alone. In the formaldehyde alone group, there was a significant reduction in the activities of SOD, CAT and GSH-Px, while curcumin could significantly prevent the impaired enzymatic activity induced by formaldehyde. Also, there was a significant increase in the renal tissue XO activity in the formaldehyde group as compared to controls, while curcumin significantly reduced the enzyme activity. Tissue NO level was significantly higher in the formaldehyde group, and curcumin significantly reduced tissue NO levels.

DISCUSSION
To our knowledge, this study is the first of its kind to assess the protective effects of curcumin against formaldehyde-induced renotoxicity in rats. In this study, parameters of oxidative stress as well as histopathological changes were assessed in the kidney tissues.

Formaldehyde exposure in rats resulted in marked dilation of the collector tubules of the rat kidney as well as dilatation of the proximal and distal tubules, and significant PAS positivity in the basal membranes of the distal tubuli. These findings clearly show the functional tissue loss induced by formaldehyde. Although the mechanisms of renotoxicity due to formaldehyde exposure are not well understood, oxidative stress is thought to play a role. Accordingly, there was a significant increase in the activity of XO enzyme in the formaldehyde exposure group that is responsible for the production of superoxide anion radicals. Similar to our results, Gulec *et al* found increased XO activity in the liver tissue of rats exposed to formaldehyde (16). Lu *et al* reported increased activity of D amine oxidase enzyme, which is an important oxidative enzyme and which is responsible for hydrogen peroxide production, upon formaldehyde exposure (17). Lino-dos-Santos-Franco *et al* in their cell culture studies found that formaldehyde induced an increase in the production of hydrogen peroxide, which is an extremely diffusible

Table 1:   The results of biochemical parameters (SOD, CAT, GSH-Px)

Groups	SOD (ng/mg protein)	CAT (k/g protein)	GSH-Px (ng/mg protein)
Group 1: control (n = 7)	0.012 ± 0.001	166.478 ± 18.389	0.084 ± 0.011
Group 2: formaldehyde (n = 7)	0.008 ± 0.001	109.332 ± 9.935	0.055 ± 0.007
Group 3: formaldehyde + curcumin (n = 7)	0.010 ± 0.002	136.150 ± 16.431	0.068 ± 0.010
	p values		
Group 1–2	0.001	0.001	0.001
Group 1–3	0.004	0.004	0.020
Group 2–3	0.004	0.011	0.042

SOD = superoxide dismutase, CAT = catalase, GSH-Px = glutathione peroxidase.
Results were expressed as mean ± standard deviation.

Table 2:   The results of biochemical parameters (MDA, XO, NO)

Groups	MDA (nmol/g protein)	XO (U/g protein)	NO (μmol/g protein)
Group 1: control (n = 7)	7.579 ± 0.828	4.211 ± 0.909	0.098 ± 0.027
Group 2: formaldehyde (n = 7)	12.104 ± 1.231	6.644 ± 1.220	0.229 ± 0.041
Group 3: formaldehyde + curcumin (n = 7)	8.294 ± 0.830	4.987 ± 0.809	0.127 ± 0.026
	p values		
Group 1–2	0.001	0.001	0.001
Group 1–3	N.S.	N.S.	N.S.
Group 2–3	0.001	0.016	0.001

MDA= malondialdehyde; XO = xanthine oxidase; NO = nitric oxide, N.S. = not significant

oxidative molecule (18). Also, the same authors reported a formaldehyde-related increase in cyclooxygenase 1 enzyme, which is an important oxidative enzyme (18). Also, they observed increased levels of IL-6 and IL-1 beta, which are important inflammatory cytokines for oxidative stress (18). MacAllister *et al* found increased oxidation due to formaldehyde through the Fenton-like system, which was reported to be a significant factor in cytotoxicity (19). Oxidative compounds such as the superoxide anion radical or hydrogen peroxide oxidize the unsaturated fatty acids, particularly those in the cell membrane, and trigger a chain reaction referred to as the lipid peroxidation. The oxidized lipids are degraded until the end-oxidation products such as MDA. This process leads to the loss of normal physiological functions of the cell and eventually to cell death. In our group, significantly increased renal MDA levels were found in the formaldehyde group, suggesting that formaldehyde may cause tissue damage through oxidative stress. Also, previous reports showed that formaldehyde may oxidize other structural and vital molecules such as certain other proteins and nucleic acids, in addition to lipids (20–22).

The results of the histopathological assessment are supportive of biochemical results. Tissue MDA levels and XO activity were lower in the formaldehyde + curcumin group as compared to the formaldehyde group.

These results suggest that curcumin was able to block the production of oxidative molecules, thus protecting the lipids of the cell membrane from oxidative stress. Also, histopathological findings of the renal tissues in the curcumin group are supportive of this view. Light microscopic examination showed that curcumin treatment was able to improve the degenerative changes in the kidney, with a histological appearance similar to than in controls, suggesting a renoprotective effect. The significant reduction in both MDA and XO levels in conjunction with microscopic findings indicates a protective effect of curcumin on formaldehyde-induced oxidative injury. Our findings are also consistent with previous reports. For instance, curcumin has been reported to provide protection against the effect of a number of different oxidative molecules (21, 23, 24). Zararsiz *et al* found a statistically significant increase in MDA activity in the kidney, after intraperitoneal formaldehyde administration (25). Ugur *et al* and Sahin *et al* concluded that curcumin may have a role in alleviating the cisplatin-induced nephrotoxicity in rats (26, 27). Sun *et al* found that curcumin was able to prevent the inflammatory response in diabetic nephropathy in a rat model (28). All these results suggest that curcumin may be effective in the removal/scavenging of the oxygen radicals

and reactive oxygen species (ROS) in the medium, thus preventing lipid oxidation.

The cellular antioxidant defence mechanisms against the noxious effects of oxygen species include enzymes such as SOD, CAT, GSH-Px and non-enzymatic compounds such as vitamin E, selenium and glutathione. These antioxidant systems detoxify or clear the ROS, protecting the cells against oxidative injury. In our study, administration of formaldehyde into the rats resulted in an impaired antioxidant defence in the renal tissues, as shown by a significant reduction in the activity of antioxidant enzymes such as SOD, CAT and GSH-Px in the formaldehyde group as compared to controls. Previous studies also showed a reduction in the antioxidant enzyme activity in rats exposed to formaldehyde (29, 30), with a lowering in the levels of other antioxidants such as selenium and glutathione (31, 32). In the curcumin group, the negative impact on antioxidant enzyme activity/levels due to formaldehyde was prevented. These results show that curcumin could prevent the impairment in the antioxidant defences caused by formaldehyde. Accordingly, curcumin group had higher tissue antioxidant levels as compared to formaldehyde alone group. Several studies are consistent with our study in terms of the results of antioxidant enzyme activity. The decrease in the antioxidant enzyme activity due to certain toxic compounds was prevented by curcumin (33, 34). These findings point out to the preventive effect of curcumin on the consumption of antioxidant enzymes or on the loss of activity.

Nitric oxide is a molecule synthetisized from arginine through a reaction catalysed by the enzyme nitric oxide synthase. It reacts with the superoxide anion radical and converts it into peroxynitrite. Peroxynitrite is an oxidative molecule, causing cellular damage, particularly via the oxidation of structural molecules such as proteins or DNA. Increased synthesis of NO and superoxide anion radicals leads to increased concentrations of peroxynitrite, causing further damage. In this study, rats in the formaldehyde group had higher NO than controls. Formaldehyde is thought to increase NO synthesis through iNOS (inducible nitric oxide synthase) activity. In the study by Ucmakli *et al* formaldehyde resulted in the increased expression of iNOS in rat hepatocytes as well as elevating the nitric oxide levels in hepatic tissues (35). Lino-dos-Santos-Franco *et al* exposed allergic rats to inhalational formaldehyde and observed increased gene expression of iNOS (36). These and similar studies show that formaldehyde causes nitrosative stress and tissue injury through increased NO synthesis caused by elevated iNOS activity. In our study, rats in the formaldehyde group had increased renal NO, while a lower NO level was found in formaldehyde + curcumin group as compared to formaldehyde alone. Many studies showed that curcumin was able to decrease the NO synthase production in activated macrophages (37) and prevented the production of NO (38, 39). Curcumin prevents the formation of peroxynitrite by its radical scavenging activity and also suppresses the NO synthesis through the inhibition of iNOS activity. Thus, we believe that it prevents cellular damage by the inhibition of nitrosative stress through the abovementioned two mechanisms.

In conclusion, in formaldehyde-induced renal injury characterized by increased renal MDA, NO and XO as well as reduced antioxidant enzyme levels, oxidative stress seems to play a major role, while curcumin treatment may have beneficial effects in terms of the prevention of the formaldehyde-induced oxidative nephrotoxicity both through histological improvement at the renal tissue level and through increased antioxidant enzyme levels and reduced oxidative products.

AUTHORS' NOTE
The authors declare that there are no conflicts of interest.

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