

RESEARCH ARTICLE

Preventive Effect of Hydrazinocurcumin on Carcinogenesis of Diethylnitrosamine-induced Hepatocarcinoma in Male SD Rats

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Abstract

The purpose of the present study was to evaluate the preventive effects of hydrazinocurcumin (HZC) on diethylnitrosamine (DEN)-induced hepatocarcinogenesis in a male Sprague Dawley (SD) rat model. One hundred and twenty male SD rats used in this study were divided into six groups. Those receiving DEN with curcumin (CUR) or HZC were studied compared with the DEN-alone group. The study demonstrated that DEN induced severe histological and immunohistochemical changes in liver tissues, significantly increasing the levels of liver marker enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -glutamyltransferase (GGT) and total bilirubin level (TBL)). The hepatocarcinoma incidences were 100.0%, 36.7% and 20.0% in the DEN-alone, DEN-CUR and DEN-HZC groups, respectively. Although macroscopic and microscopic features suggested that both CUR and HZC were effective in inhibiting DEN-induced hepatocarcinogenesis, HZC was exerted a stronger influence. Immunohistochemical analysis with PCNA demonstrated significant differences among the groups (all $P < 0.05$). Taken together, the results suggested application of CUR and HZC could prevent the occurrence of carcinogenesis and HZC may be a more potent compound for prevention of DEN-induced hepatocarcinogenesis in rats than CUR.

Keywords: Hepatocarcinoma - diethylnitrosamine - hydrazinocurcumin - curcumin

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Introduction

Hepatocarcinoma is one of the major malignant tumors in humans, representing the fifth most prevalent malignancy worldwide and the third leading causes more than 250000 deaths annually (Farazi et al., 2006). Owing to advances in diagnostics and therapeutics, hepatocarcinoma can be curatively treated when detected at an early stage by applying therapies including surgical resection, radiofrequency ablation (RFA) and transcatheter arterial chemoembolization (TACE). However, curative treatment are often hampered by frequent recurrence of hepatocarcinoma (Okuda, 2007), because the remaining liver retains the potential for carcinogenesis (Kumada et al., 1997). Effective measures of chemoprevention represent an important hope for people living in these areas. To our disappointment, no chemotherapy agent has better benefit in controlled clinical trials and treatment outcome due to different kinds of reasons including drug resistance and toxicity to normal cells (Nagano, 2010).

Curcumin (CUR), the active component of turmeric has potent anti-tumor effects to hepatocarcinoma in clinical trials (Dai et al., 2013) and several other cancers, such as colon cancer (Lee et al., 2009) and breast cancer (Mehta et al., 1997). Although CUR is remarkably non-

toxic and has promising anti-cancer activities, preclinical and clinical studies indicated that its poor bioavailability and pharmacokinetic profiles due to its instability under physiological conditions had limited its application in anti-cancer therapies (Anand et al., 2007; Appiah-Oppong et al., 2008).

In an attempt to retain CUR's favorable medicinal properties and safety profile while increasing its potency, one pyrazole analogue of curcumin (hydrazinocurcumin, HZC) was synthesized and applied to many cancer cell types. It was obtained as pale yellow gum which was analyzed for C₂₁H₂₀N₂O₄ by HRMS (Shim et al., 2002). Ishida found HZC had a broad cytotoxicity spectrum in all cancer cell lines, especially HepG2, which the ED₅₀ values was the lowest in all curcumin analogues (Ishida et al., 2002). Wang showed that compared with CUR, HZC was more effective in inhibiting STAT3 phosphorylation and down-regulating an array of STAT3 downstream targets which contributed to the suppression of cell proliferation, colony formation, depressing cell migration and invasion as well as induction of cell apoptosis (Wang et al., 2002).

Rathore reported a validated High Performance Liquid Chromatography (HPLC) method for simultaneous determination of HZC and phenol red in intestinal perfusate along with different pH for further pharmaceutical

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development (Rathore et al., 2008). It possesses a favourable intestinal permeability in comparison with CUR. Compared with CUR, HZC has greatly improved water solubility and stability, and has high cell permeability, anti-tumor activity, improved bioavailability with more favorable pharmacological activity. Up to now, HZC has been used for some different medical purposes in vitro, however there is no study that demonstrated the anti-hepatocarcinoma efficacy of HZC by models in vivo. Whether HZC has potential as a new therapeutic agent for hepatocarcinoma to same extent as CUR remains to be investigated. In our study, we focused on the effect of HZC on hepatocarcinoma induced by DEN in male SD rat model.

Materials and Methods

Drug preparation

HZC was synthesized as described by CUR, CUR and DEN were obtained from Sigma-Aldrich Corp (St. Louis, MO, USA). All other chemicals and reagents used were analytical grade. Proliferating cell nuclear antigen (PCNA) was purchased from Zymed Company (Carlsbad, CA, USA). The rest of the chemicals utilized were purchased from Beijing Zhongshan Biotechnology Co, Ltd.

Animals

Male SD rats weighting 100–120 g were provided by the Animal Center of Hebei Medical University. All animals were received humane care and protocols were approved by the Hebei Medical University Animal Ethics Committee. They were acclimatized to animal house conditions (an air-ventilated room under a 12h light/dark cycle with constant temperature (23°C) and humidity (55%)) and were fed with commercial pelleted rat chow and water throughout the experimental period of 25 weeks (including 1 week of acclimatization).

Treatments

Rats were randomised divided into six groups accordance with the requirements after 1 week of acclimatisation. Group 1 (control non-treated group) was considered as normal control group. Rats in group 2 (DEN-bearing non-treated group) were given intraperitoneal injection of DEN (50 mg/kg) twice a week for 12 weeks. CUR in group 3 (control CUR-treated group) was administered intraperitoneally (80 mg/kg) twice a week for 12 weeks. Rats in group 4 (DEN-bearing CUR-treated group) were administered with both DEN as in group 2 and CUR as in group 3. HZC in group 5 (control HZC-treated group) was administered intraperitoneally (80mg/kg) twice a week for 12 weeks. Rats in group 6 (DEN-bearing HZC-treated group) were administered with both DEN as in group 2 and HZC as in group 5.

Body weight was recorded at the end of every week for 24 weeks. The experiment was terminated at the end of 25 weeks (including 1 week of acclimatization) and all the surviving living rats were anaesthetized and sacrificed at the end of the experiment following animal ethic guidelines. Blood samples were taken from all groups via cardiac puncture before sacrifice. The blood

Table 1. Effect of CUR and HZC on the Survival and Development of Nodules on DEN-induced Hepatocarcinogenesis in Rats

Group	Treatment	Survival(%)	tumor incidence(%)	Average count of nodules/nodules bearing liver
1	Control	100(10/10)	—	—
2	DEN alone	60(18/30) ^a	100% (30/30) ^{aaa}	17.17±1.08 ^{aaa}
3	CUR alone	100(10/10)	—	—
4	DEN+CUR	83.3(25/30) ^b	36.7%(11/30) ^{bbb}	13.36±2.17 ^{bbb}
5	HZC alone	100(10/10)	—	—
6	DEN+HZC	93.3(28/30) ^{bb}	20.0%(6/30) ^{bbb}	11.83±3.90 ^{bbb}

Values of results are expressed as Mean±S.D. ^a*P*<0.05, ^{aa}*P*<0.01, ^{aaa}*P*<0.001 (significantly compared with control group); ^b*P*<0.05, ^{bb}*P*<0.01, ^{bbb}*P*<0.001 (significantly different from DEN alone group)

samples were centrifuged at 2500 rpm for 10 min, serum was then separated and kept at –20 °C until used for biochemical assays. Determination of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -glutamyltransferase (GGT), total bilirubin level (TBL) were measured by automatic biochemical analyzer. Liver was excised immediately and then counted tumors, observe tumor morphology. Meanwhile body and liver weight were measured.

Cytomorphologic Evaluation and Immunohistochemistry

Autopsy specimens were taken from the liver of rats in all groups. All the liver tissues were fixed routinely, and embedded in paraffin to prepare 4 μ m sections for haematoxylin and eosin (H&E) staining. The diagnosis and grade of hepatocarcinoma was established based on the morphologic findings identified on the cell block sections using the World Health Organization criteria (Theise et al., 2010). The expression strength of PCNA was based on both the staining intensity (0, negative; 1, weak; 2, intermediate; 3, strong) and the percentage of positive cells (0, 0% cells; 1, \leq 25% positive cells; 2, 26–50% positive cells; 3, >50% positive cells). The two scores were added, with a maximum score of 6. A score of >2 represented a positive immunohistochemical identification of a marker.

Statistical analysis

Data were analyzed by the Chi-square test and rank sum test using SPSS13.0 statistical software. A value of *P* < 0.05 was considered statistically significant. The data were also expressed as mean±SD with animals in each group.

Results

In this study, SD rats were used as test animals, CUR and HZC as antitumor agents and DEN as an inducer of liver tumorigenesis in vivo. This experiment was designed to determine whether preventive effect of CUR or HZC existing on the liver tumorigenesis. Data on incidence, survival rate, tumor amount, weight gain, absolute and relative weight of liver (liver/body weight×100) of hepatocarcinoma from animals belonging to various experimental groups had been compared at the end of 25 weeks of experimental period and have been presented below (Tables 1, 2).

Table 2. Effect of CUR and HZC on Body, Liver and Relative Liver Weights on DEN-induced Hepatocarcinogenesis in Rats

Group	Treatment	Final body weight (g)	Gain in body weight (g)	Liver weight (g)	Relative Liver weight (g)
1	Control	408.9±7.2	299.5±6.9	15.33±0.44	3.75±0.11
2	DEN alone	333.8±8.8 ^{aaa}	223.1±8.3 ^{aaa}	16.36±0.49 ^{aa}	4.91±0.15 ^{aaa}
3	CUR alone	404.4±7.5	295.5±8.7	15.28±0.47	3.78±0.12
4	DEN+CUR	386.2±7.0 ^{bbb}	278.5±7.5 ^{bbb}	15.72±0.29 ^b	4.08±0.12 ^{bbb}
5	HZC alone	403.8±5.2	294.7±6.0	15.30±0.37	3.79±0.09
6	DEN+HZC	386.8±5.2 ^{bbb}	280.9±2.7 ^{bbb}	15.64±0.29 ^b	4.04±0.08 ^{bbb}

Values of results are expressed as Mean±S.D. ^a*P*<0.05, ^{aa}*P*<0.01, ^{aaa}*P*<0.001 (significantly compared with control group); ^b*P*<0.05, ^{bb}*P*<0.01, ^{bbb}*P*<0.001 (significantly different from DEN alone group)

Table 3. Effect of CUR and HZC on ALT, AST, ALP, GGT and TBL on DEN-induced Hepatocarcinogenesis in Rats

Group	Treatment	ALT(U/L)	AST(U/L)	ALP(U/L)	GGT(U/L)	TBL(mg/dL)
1	Control	50.0±5.5	96.7±7.1	92.5±8.0	10.0±1.3	0.73±0.06
2	DEN alone	284.4±14.2 ^{aaa}	398.3±14.8 ^{aaa}	237.1±7.8 ^{aaa}	130.1±9.0 ^{aaa}	1.67±0.06 ^{aaa}
3	CUR alone	60.7±5.25	106.4±7.5	96.5±5.0	10.7±1.1	0.76±0.06
4	DEN+CUR	125.32±8.2 ^{bbb}	222.7±7.4 ^{bbb}	164.8±5.4 ^{bbb}	51.8±4.1 ^{bbb}	0.98±0.03 ^{bbb}
5	HZC alone	63.5±4.0	107.1±8.7	99.3±5.5	10.7±1.1	0.78±0.06
6	DEN+HZC	132.6±6.3 ^{bbb}	235.7±10.6 ^{bbb}	171.6±6.6 ^{bbb}	55.9±3.7 ^{bbb}	1.10±0.05 ^{bbb}

Values of results are expressed as Mean±S.D. ^a*P*<0.05, ^{aa}*P*<0.01, ^{aaa}*P*<0.001 (significantly compared with control group); ^b*P*<0.05, ^{bb}*P*<0.01, ^{bbb}*P*<0.001 (significantly different from DEN alone group)

Table 4. Expression of PCNA on DEN-induced Hepatocarcinogenesis in Rats

Group	Treatment	PCNA protein-positive tumors (%)
1	Control	0.0
2	DEN alone	70.0 ^{aaa}
3	CUR alone	0.0
4	DEN+CUR	26.7 ^{bb}
5	HZC alone	0.0
6	DEN+HZC	13.3 ^{bbb}

Values of results are expressed as Mean±S.D. ^a*P*<0.05, ^{aa}*P*<0.01, ^{aaa}*P*<0.001 (significantly compared with control group); ^b*P*<0.05, ^{bb}*P*<0.01, ^{bbb}*P*<0.001 (significantly different from DEN alone group)

Liver tumor formation

After 25 weeks of feeding, 19 of the 120 experimental rats died: 12 in the DEN-bearing non-treated group, 5 in the DEN-bearing CUR-treated group, 2 in the DEN-bearing HZC-treated group. The observed survival rate in DEN-bearing non-treated group was significantly lower compared with the control non-treated group (Table 1). Although survival rate in groups of rats receiving DEN with CUR or HZC had no statistical differences, significant improvement in survival was observed when compared with DEN-bearing non-treated group. No mortalities were observed in group of control non-treated as well as those receiving CUR or HZC control group for 25 weeks of experimental period.

No hepatocarcinoma rat was found in three control groups. The incidence of rat was 100.0%, 36.7% and 20.0% in DEN-bearing non-treated, DEN-bearing CUR-treated and DEN-bearing HZC-treated group, respectively. The incidence of hepatocarcinoma was significantly lower in the DEN-bearing CUR-treated and DEN-bearing HZC-treated group than in the DEN-bearing non-treated group (all. *P* < 0.05) (Table 1). As can be seen in tumour incidence by comparing, HZC can inhibit tumorigenesis better than CUR. The hepatocarcinogenic rats were

infiltrated with a large number of nodules, resulted in making the precise assessment of tumor mass impossible. The literature showed that nodules larger than 6 mm in diameter at week 24 were always hepatocarcinoma (Taras et al., 2007), so we quantified these nodules according to this standard when detectable at the surface of the liver. No hepatic nodule was observed in control non-treated, control CUR-treated or control HZC-treated group. In the surviving DEN-treated rats, the average count of nodules was 17.17 ± 1.08, while it was only 13.36±2.17, 11.83 ± 3.90 in the DEN-bearing CUR-treated and DEN-bearing HZC-treated group respectively (all. *p* < 0.05) (Table 1). Although there was no statistical significance between DEN-bearing CUR-treated and DEN-bearing HZC-treated group, they all declined count of nodules significantly in hepatocarcinoma compared with DEN-bearing non-treated group. As another marker of primary tumor mass, the liver mass of DEN-bearing non-treated rats increased in size and number resulted in an increased liver/body weight ratio which had significant difference compared with control non-treated group (4.91±0.15% vs 3.75±0.11%, *p* < 0.05). We measured the liver/body weight ratio which was significantly lower in rats treated with CUR or HZC than in DEN-bearing non-treated group (4.91±0.15% vs 4.08±0.12%, *p* < 0.05; 4.91±0.15% vs 4.04±0.08%, *p* < 0.05). The comparison results of absolute liver weight in various groups were the same as contrast of liver/body weight ratio. The absolute and relative liver weights were higher in the DEN-bearing non-treated group than in the DEN-bearing CUR-treated or DEN-bearing HZC-treated group, but no significant differences were observed between the latter two groups. CUR and HZC administration during DEN treatment appeared to protect remarkably against DEN-induced loss in body weight and increase in liver weight. Although weight gains in DEN-bearing non-treated group reduced remarkably, similar weight gained in animals of various groups. The mean weight of DEN-bearing CUR-treated and DEN-bearing

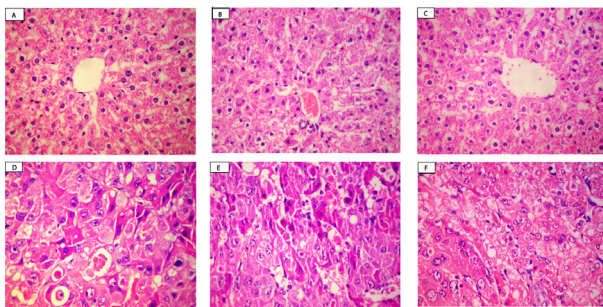


Figure 1. Observation of Histopathology in Rat Liver(Original Magnification: 400×): (A) Liver tissue from the normal group showed hepatic lobules with normal architecture; (B) Liver tissues from rats treated with CUR only indicated moderate to severe venous and sinusoidal congestion; (C) Liver tissues from rats treated with HZC only showed less venous and sinusoidal congestion than the group of treated with CUR only; (D) Liver tissue from rats in DEN-bearing non-treated group showed cancer focus; (E) Hepatic tissues from rats injected with DEN and treated with CUR showed cancerous focus with focal necrosis; (F) Hepatic tissues from rats injected with DEN and treated with HZC showed cancerous focus with patchy necrosis

HZC-treated rats remained stable whereas decreased from the 15 week in DEN-bearing non-treated rats. At the time of sacrifice, there was an appreciable increase in the body weight in DEN-bearing CUR-treated and DEN-bearing HZC-treated rats compared with rats given DEN alone ($223.1 \pm 8.3\text{g}$ vs $278.5 \pm 7.5\text{g}$, $p < 0.05$; $223.1 \pm 8.3\text{g}$ vs $280.9 \pm 2.7\text{g}$, $p < 0.05$).

Biochemical assays

The index of liver function in control non-treated group was normal. Rats from CUR and HZC showed an insignificant increase in liver enzymes activities in comparison to control non-treated group. The serum from rats in DEN-bearing non-treated group showed a highly significant increase in levels of liver enzymes (ALT/AST/ALP/GGT/TBL) when compared to those from control non-treated group probably reflecting hepatic injury induced by DEN (Table 3). Though the serum from DEN-bearing CUR-treated and DEN-bearing HZC-treated groups displayed increase in levels of liver enzymes when compared to those from control non-treated group, both groups expressed a significant decrease in comparison to those from DEN-bearing non-treated group. Although receiving DEN with CUR or HZC did not show statistical difference in inhibiting the DEN-induced elevation of liver function, the protective effect of liver function in latter group was more superior. These data imply a promising result that HZC has preventive effect on DEN-induced hepatocarcinogenesis.

Pathologic examination of hepatocarcinoma with HE staining

The histological sections of livers from control non-treated group (group 1) displayed the normal organization of hepatic lobules consisting of one to two-cell-thick hepatic cords radiating from a central vein towards the lobular periphery. The hepatic cords bordered irregular and endothelium-lined hepatic sinusoids (Figure 1a). Although

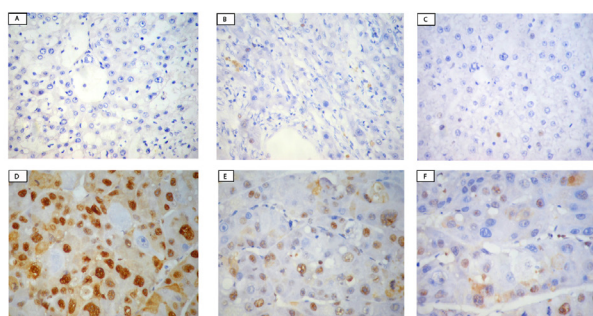


Figure 2. Immunohistochemical Staining of PCNA in Rat Liver(Original Magnification: 400×): (A) Liver from normal group showing no PCNA staining pattern; (B) Hepatic tissues from rats treated with CUR showing very few PCNA-staining; (C) Hepatic tissues from rats treated with HZC showing less PCNA-staining cell as well; (D) Liver from rats in positive control group showing some colonies of strong PCNA-staining; (E) Hepatic tissues from rats injected with DEN and treated with CUR displaying moderate PCNA-immunostaining; (F) DEN-bearing HZC-treated group showing more milder PCNA-staining than DEN-bearing CUR-treated group

liver tissues from rats treated with CUR only (group 3) showed no significant difference to the group of control non-treated (group 1), it displayed moderate to severe venous and sinusoidal congestion (Figure 1b). Compared with group 3, liver tissues from rats treated with HZC only (group 5) also had the normal lobular organization represented by central vein, hepatic cords and sinusoids, but showed less congestive (Figure 1c). DEN alone (group 4) showed loss of architecture, hepatic parenchyma with thick cords of polyhedral and more mitotic cells with granular cytoplasm (Figure 1d). Compared with DEN-bearing non-treated group (group 2), cells in DEN-bearing CUR-treated group (group 4) were moderately malignant which showed focal necrosis and less mitotic count (Figure 1e). DEN-bearing HZC-treated group (group 6) showed cancerous focus with patchy necrosis (Figure 1f).

Immunohistochemical examinations

PCNA was expressed in the nuclei of hepatic cells. The positive tumor for PCNA was significantly lower in the DEN treated with CUR or HZC group than in DEN-bearing non-treated group (all. $P < 0.05$). Photomicrographs of PCNA-immunohistochemical sections of various groups were showed (Figure 2).

Discussion

Liver is a major organ in which most of the chemicals, drugs and carcinogens undergo metabolism (Devasena et al., 2002). Animal models can be used to simulate carcinogenesis and development of human hepatocarcinoma and to study its molecular mechanism and intervention (Kang, 2012). SD rat has been widely accepted to be very similar to human hepatocarcinoma, making this model ideal for hepatocarcinoma studies (Sreepriya et al., 2005). DEN is one of the several environmental carcinogens have been reported to elicit hepatic tumorigenesis (Gayathri et al., 2009). DEN can be used as an initiating agent in two stage (initiation and

promotion) protocols for hepatocarcinogenic studies (Park et al., 2001).

The course of hepatocarcinoma rat model induced by a low dose of DEN discontinuously is similar with the generating process of human primary hepatocarcinoma. In addition, the rate of induced hepatocarcinoma is higher and the mortality is lower than a single megadose of DEN (Hasegawa et al., 1991). The results of the present study provide support for the preventive effects of HZC and CUR against DEN-induced hepatocarcinogenesis in rats. Decreased appetite, consequently reduction in food intake and the loss of body weight observed in the hepatocarcinoma induced by DEN could be an indirect indication of the declining hepatic function following exposure to hepatocarcinogen (Farazi et al., 2006). Previous studies have demonstrated that the hepatocarcinoma results in a rapid and progressive body weight loss and tissue waste, particularly evident for the skeletal muscle and adipose tissue with relative sparing of visceral proteins, this depletion being mainly accounted for by accelerated protein catabolism (Tessitore et al., 1993).

Greaves reported an increase in the liver weight of the animals following exposure to DEN, which could be attributed to the formation of nodules and tumors in the liver following carcinogen exposure (Greaves et al., 1986). In accordance with the above report we also observed an decrease in body weight and increase in liver weight in animals administered with DEN. PCNA as a index of reflecting state of cell proliferation and judgment of malignant degree in the nucleus of hepatocarcinoma enhanced significantly, which has been confirmed in many studies (Kelloff et al., 1999). So enhanced expression of PCNA indicates abnormal proliferation of liver tissue. Our results showed that DEN treatment led to increased expression of PCNA in SD rats, which suggested DEN significantly induced the development of hepatocarcinoma. The DEN-induced hepatocarcinogenesis in our rat study model was confirmed the preventive effect of HZC as seen by histopathological and immunohistochemical findings.

It is known to all that the activity of ALT, AST, ALP, GGT and TBL are representative of liver function and their increased levels are sensitive indicators of hepatic injury (Singh et al., 2009). Generally, liver damage induced by DEN is related to the disruption of liver cell metabolism and membrane instability and subsequently causes distinctive changes in the activities of serum enzyme. The elevation of ALT activity is credited to hepatocellular damage and is usually accompanied by a rise in AST (Al-Rejaie et al., 2009). ALP as one of the liver function enzymes is closely connected with lipid membrane in the canalicular zone. Increased level in ALP reflects pathological alteration in biliary flow. Therefore, any interference with the bile flow, whether extra-hepatic or intra-hepatic leads to increased serum level of ALP activity (Nair et al., 1998). Upon liver injury, liver marker enzymes (AST, ALT, and ALP) enter into the circulatory system because of the altered permeability of the membrane (Sivaramakrishnan et al., 2008). GGT is a membrane-bound enzyme, mainly in the canalicular domain. GGT exhibits a tissue specific expression and

modified under various physiologic and pathologic conditions, such as development and carcinogenesis (Yao et al., 2004). The significant elevation of GGT in rat sera may be attributed to the liberation of this enzyme from the plasma membrane into the circulation indicating damage of cell membrane as a result of carcinogenesis (Bulle et al., 1990). The discharge of TBL reflects pathological a nonspecific alteration in the plasma membrane integrity and/or permeability (Sivaramakrishnan et al., 2008). Marx (Marx, 1996) reported that the increase of TBL might be due to the toxic effect of carcinogen on hepatocytes and sinusoidal cells, which causes the reticulin network surrounding the central vein to collapse producing hemorrhage and increasing bilirubin formation. So we considered administration of HZC had a protective effect on the liver by decreased activities of ALT, AST, ALP, GGT and TBL.

COX-2 is a isozyme induced rapidly by both inflammatory and mitogenic stimuli (Verbarg et al., 2001). COX-2 signaling influence the carcinogenesis through inflammation suppression, immune response suppression, apoptosis inhibition, angiogenesis regulation and tumor cell invasion (Müller-Decker et al., 2011), which are all crucial in the development and progression of cancer (Wang et al., 2012). Cellular expression of COX-2 is increased in the earliest stage of carcinogenesis through tumor development and invasive tumor growth (Yildirim et al., 2008). Several cell lines of evidence suggest that COX-2 signaling is implicated in hepatocarcinogenesis. COX-2 inhibitors prevent hepatocellular carcinoma cell growth in vitro and in animal models (Kern et al., 2004). Previous research demonstrated that COX-2 expression enhanced malignant transformation induced by DEN in hepatocytes of a model of transgenic mice (Llorente et al., 2011). COX-2 is widely regarded as a potential pharmacological target for preventing and treating inflammatory and cancer diseases. So therapeutic strategies have focused primarily on selective inhibitors of COX-2 activity. Since the promoter region of COX-2 contains two nuclear factor- κ B (NF- κ B) binding sites, and TNF α is a potent activator of NF- κ B in many cell types. So CUR causes cancer prevention through interfering with the activity of NF- κ B (Huang et al., 1991) and suppression of expression of the enzyme in COX-2 (Plummer et al., 1999). Selvam (Selvam et al., 2005) designed an experiment which pyrazole analogues (including HZC) of CUR were synthesized and evaluated for COX-2 inhibitory, antioxidant and anti-inflammatory activities. They found that HZC significantly enhanced COX-2 selectivity and possessed significant anti-inflammatory activity in carrageenan induced rat paw edema assay compared with CUR. The studies revealed that replacement of the β -dikete fragment of CUR by a pyrazole ring significantly enhances COX-2 selectivity. HZC showed higher anti- COX-2 activity than CUR because of possessing two methoxy groups. We considered that the molecular mechanisms of the action of HZC were possibly by inhibiting the NF- κ B pathway, coupled with the reduction of the expression of NF- κ B regulated genes in COX-2. We may conclude that HZC provide superior anti-tumor activity by suppressing expression of COX-2 which interfered with the activity of NF- κ B.

In this study, we divided SD rats into six groups, DEN-bearing non-treated, DEN-bearing CUR-treated and DEN-bearing HZC-treated group according to requirement of experimental design which were injected DEN into peritoneum to induce hepatocarcinoma. A series of indexes of hepatocarcinoma were recorded for each group to judge the protective role of CUR and HZC against hepatocarcinoma in rats. Our results showed that CUR and HZC decreased sensitivity of rats to DEN, resulting in decreased incidence, tumor amount, absolute and relative weight of liver, liver marker enzymes and increased survival, weight gain, the HZC was better.

In summary, based on our preliminary experiment, we suggest preventive effect of HZC against DEN-induced hepatocarcinogenesis in SD rats. Although this study adds a therapeutic trial with this drug in SD rats, the detailed mechanism of action still remains to be investigated.

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