Research Article

Of Bulk and Nano: Comparing the Hepatoprotective Efficacy of Curcumin in rats

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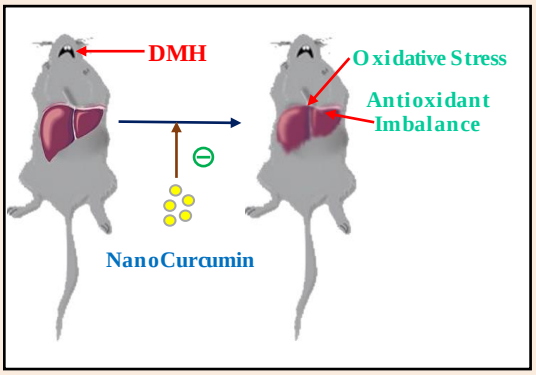
Abstract

We have recently optimized the synthesis and antioxidant dose of curcumin nanocrystals and proved its protective effect against red cell toxicity in mammalian model. Humans are exposed to dimethyl hydrazine (DMH) and other hydrazines through environment. DMH metabolites alter the antioxidant status and stimulates oxidative stress. Hence, our present study investigated the effects of optimized dose of curcumin nanocrystals in 1,2-dimethyl hydrazine-treated rats by evaluating the oxidative stress. Our study reveals that curcumin nanocrystals mediate its hepatoprotective effect by decreasing lipid peroxidation, and by enhancing the activities of antioxidant enzymes (superoxide dismutase, catalase), GSH content and GSH-dependent detoxification enzymes (glutathione peroxidase, glutathione-S-transferase). This may be due to enhanced solubility, dispersibility, and crystallinity of the nanocrystals, which might have enhanced its bioavailability when compared to poorly soluble, bulk curcumin. We conclude that curcumin nanocrystals could be used to ameliorate the hepatic oxidative stress associated with several illness and toxicity.



Keywords: Hepatotoxicity, curcumin nanocrystals, oxidative stress, antioxidant

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Introduction

Metabolism of toxic components such as drugs, carcinogens, mutagens and pollutants takes place in liver, the major detoxifying organ [1]. Hepatic biochemical changes could therefore be used as markers of chemical toxicity and as indicators of antitoxicability of antioxidants and antitumor drugs. Previous reports have also highlighted the significance of hepatic biochemical changes in monitoring the carcinogen-induced toxicity and efficacy of antioxidants [2,3,4,5]. 1,2-Dimethylhydrazine (DMH) is a toxic environmental pollutant and a procarcinogen [6,7]. Humans are exposed to DMH and other hydrazines through environment. DMH is metabolized into electrophilic diazonium ion that is known to elicit oxidative stress, thus altering the antioxidant balance in the liver [8]. Therefore, DMH can also be considered as a hepatotoxic agent. The markers of oxidative stress such as lipid peroxidation, enzymic and non-enzymic antioxidants may be the indicators of DMH-induced hepatotoxicity. These biochemical changes not only interpret the severity of the toxicity but also the antioxidant and detoxification efficacy of therapeutics. For example, we have used hepatic biochemical changes as the cancer and cancer-therapeutic markers in DMH-administered rats treated with curcuminoids (Curcumin and Bisdemethoxy curcumin analog) as a chemotherapeutic [4].

Curcumin was reported to be beneficial in preventing hepatic toxicity during colon cancer [4] and also in ameliorating oxidative stress [9]. Furthermore, curcumin possess no side effects even after long term administration in laboratory animals [10]. In spite of these advantages, curcumin holds certain disadvantages like poor intestinal

absorption, pharmacologically insignificant accumulation in tissue, reduced dissolution and solubility in physiological pH and meager bioavailability [11]. Previous studies on rats and human suggested that, even at a dose of 12 g/kg body weight curcumin showed poor bioavailability [10]. Nanotisation of curcumin to form curcumin nanocrystals (CNC) is the best approach to get better bioavailability. When drugs are formulated into nanocrystals, they possess major advantages: i) possibility to administer through any route ii) possibility to form orally ingestible nanosuspensions iii) enhancement in active surface area iv) enhancement in saturation solubility [12]. We have recently optimized the synthesis and antioxidant dose of curcumin nanocrystals (40 mg/kg body weight) and proved its attenuating effect against DMH-induced circulatory toxicity [13]. We have also reported that low dose of nanotized curcumin nanocrystals are more potent antioxidant than high dose of bulk curcumin. In continuation of our previous investigation, our present study aims to evaluate the hepatotoprotective effect of the optimized dose of curcumin nanocrystals [13] during DMH-induced toxicity.

Experimental

Animals

Male albino rats of Wistar strain weighing between 100 and 120 g were obtained from the King's Institute Chennai after Institutional Animal Ethical Committee approval. Animals were housed in polypropylene cages. Commercial pellet feed and water were given *ad libitum*.

Chemicals

DMH, bovine serum albumin, and other fine chemicals were obtained from Sigma Chemical Company, St. Louis, USA. All other chemicals and reagents used were of analytical grade.

Synthesis of curcumin nanocrystals

Curcumin nanocrystals (CNC) of 25 nm size were prepared by precipitation method as reported earlier [13].

Animal Experimental protocol

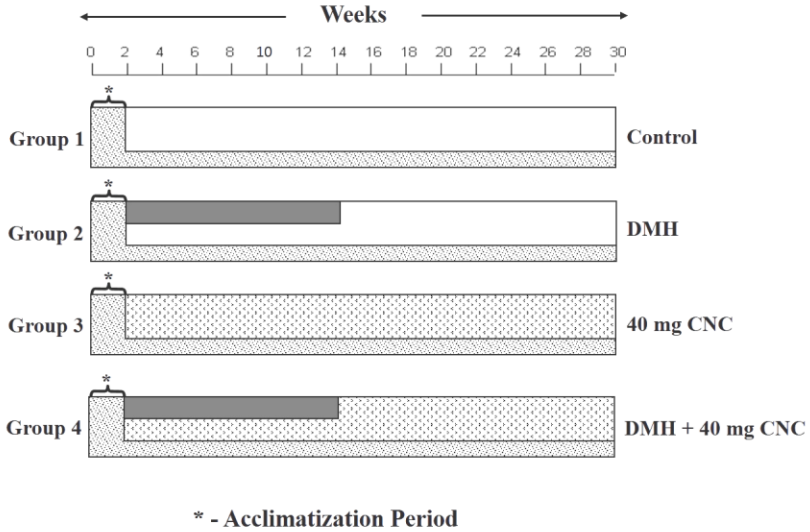

Figure 1 Experimental groups

Rats were initially randomised into four groups (n=12) as shown in **Figure 1**. After 2 weeks of acclimatization, rats in group 1 served as a normal control. Rats in group 2 received a weekly subcutaneous injection of DMH (20 mg/kg

body weight) in the groin for 15 weeks [8]. Rats in group 3 received curcumin nanocrystals (CNC) (40 mg/kg body weight), daily for 30 weeks through intragastric route. Rats in group 4 were administered with both CNC as in group 2 and DMH as in group 1. The experiment was terminated at the end of 30th week. All animals were anesthetized after an overnight fast. Liver was excised and homogenized in phosphate buffer at physiological pH.

Liver analysis

Liver homogenate was analysed for oxidative stress parameters. Lipid peroxidation, as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was estimated by the method of Yagi [14]. Superoxide dismutase (SOD) was assayed by the method of Kakkar et al. [15] and catalase (CAT) was assayed by the method of Sinha [16]. Reduced glutathione was estimated by the method of Ellman [17]. The activities of glutathione peroxidase (GPx) and glutathione S-transferase (GST) were estimated by the method of Rotruck et al. [18] and Habig et al. [19] respectively. Tissue protein was determined by the method of Lowry et al. [20].

Statistical analysis

The data of hepatic biochemical parameters are presented as mean ± sd. The data were analysed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if $P \leq 0.05$.

Results and Discussions

Effect of CNC on DMH-induced hepatic oxidative stress was determined by measuring Thiobarbituric acid reactive substances (TBARS, an index of lipid peroxidation) and by assaying the antioxidant enzymes (SOD and CAT), GSH content and detoxification enzymes (GPX and GST). Values of these parameters are shown in **Table 1**.

Table 1 Amount of TBARS, GSH and the activities of GPX, GST, SOD and CAT in the liver of different groups

Group	TBARS m mol/mg tissue	GSH mg/100g tissue	GPX Units [a]/mg protein	GST Units[b]/mg protein	SOD Units[c]/mg protein	CAT Units[d]/mg protein
Control	3.25 ± 0.10	344.14± 20.06	11.25±0.14	1036.23±39.84	7.40 ± 0.20	40.99 ± 3.86
DMH	6.11 ± 0.18[$]	293.26±21.62 [$]	6.12±0.17 [$]	925.58±40.65 [$]	2.6 0 ± 0.22[$]	24.22 ± 3.11[$]
40mg CNC	3.22 ± 0.12	345.26±21.00	11.26±0.16	1038.86±38.88	7.50 ± 0.25	41.01 ± 3.94
DMH+40mg CNC	3.20 ± 0.28[*]	341.66±20.09 [*]	10.90±0.20 [*]	1028.84±39.59 [*]	6.30 ± 0.50[*]	38.94 ± 3.63[*]

[$] Values differ significantly from Control group;
[*] Values differ significantly from DMH group
[a] µM of GSH utilized /min;
[b] µM of CDNB-GSH conjugate formed /min.
[c] Enzyme required for 50% inhibition of nitroblue tetrazolium reduction;
[d] µM of H_2O_2 utilized per minute

Liver TBARS level was highest in DMH-treated group. However, intragastric administration of CNC to DMH-injected rats significantly reduced the liver TBARS level to near normal values at the dose of 40 mg CNC. The dose of CNC (40 mg) does not produce any alteration in liver TBARS in normal rats (Group 3).

Hepatic GSH content and the activities of GPX, GST, SOD and CAT were all significantly reduced in the liver of DMH treated rats when compared to control rats. Values of all these parameters were however raised significantly to near normal values in DMH + 40 mg CNC group when compared to DMH treated rats. In addition, the values observed in the rats treated with the dose of CNC (40 mg) do not differ significantly from that of untreated control rats.

We have already reported the hepatoprotective effect of bulk curcumin and curcumin analog at 80 mg concentration in DMH treated rats [4]. However, the poor solubility and dispersibility of curcuminoids reduce their bioavailability, thus justifying higher doses to be ingested. This problem can be circumvented by scaling down the size of the particles to nanosize, as nanoparticles have many advantages like improved solubility, enhanced bioavailablity and low treatment dose [12]. Therefore we have recently optimized the synthesis of nanocrystals of curcumin and reported its protective effect against circulatory toxicity in DMH-treated rats, after fixing the dose to be equal to and lesser than that of the reported dose of bulk curcumin [13].

Enhanced LPO level and a concomitant fall in the GSH, GSH-dependent enzymes (GPX and GST) and antioxidant enzymes (SOD and CAT) in the liver of DMH treated rats may be due to the hepatic metabolism of DMH into diazonioum ion, that elicits oxidative stress. Earlier reports have also reported the role of metabolic product of DMH and other carcinogens in eliciting hepatic oxidative stress [8]. Our present data reveal that reactive oxygen species induces oxidative stress in the liver of DMH treated rats. Glutathione (GSH) is a primary antioxidant that is responsible for the detoxification of reactive oxygen species such as hydrogen peroxide or lipid peroxides. The toxic electrophiles and reactive oxygen species are counteracted by the antioxidant enzymes SOD and CAT for further elimination [21]. Therefore, we suggest that the rise in LPO and fall in GSH content, GPX, GST, SOD and CAT activities in the liver of DMH treated rats may be due to the utilization of hepatic enzymes to counteract the DMH-mediated oxidative stress and LPO.

Decrease in the level of LPO and restoration of GSH content, GPX, GST, SOD and CAT activities (to near normal values) in DMH treated rats ingested with 40 mg CNC clearly reveals the detoxifiying and antioxidant potentials of CNC. This observation clearly suggests that CNC is an effective hepatoprotective antioxidant. Upregulation of detoxification enzymes is reported to enhance the metabolism and disposal of toxic chemicals [22]. Therefore, we suggest that CNC prevents DMH-induced hepatic liver injury by increasing the activities of GSH-dependent detoxification enzymes. Hammed et al. [23] has reported that nanoparticles exert their detoxification effects by scavenging reactive oxygen species and by enhancing cellular detoxification enzymes. These reports are in line with our findings. It could therefore be suggested that increasing the GSH content and GSH-dependent detoxification enzyme activities is one of mechanism by which curcumin nanocrystals ameliorates DMH-induced liver injury and functions as hepatoprotecting agent.

Nanoparticles have attracted much attention as an antioxidant and anti-inflammatory agent due to their enhanced surface area and small size [24,25,26]. Increased surface area and decreased size of nanoparticles may contribute to more bioavailability [27,28]. Thus, we suggest that the CNC with a size range of 26 nm, posses more bioavailability at a dose of 40 mg. Our results confirming the detoxification and antioxidant property of CNC is consistent with those of previous reports that nanoparticles exhibit enhanced antioxidant activity due to their ability to scavenge reactive oxygen species via utilization of detoxification and antioxidant enzymes [29]. Reports suggest that nanoparticles possess higher dispersibility and faster cell entry as compared to their bulk counterparts [30]. We have also already reported that CNC shows greater dispersibility and solubility when compared to its bulk counterpart [13] which inturn may enhance its bioavailability and consequently cutting down the therapeutic dose level. Therefore, we

could infer that CNC exerts its hepatoprotective effect by restoring the activities of detoxification and antioxidant enzymes and by decreasing the LPO, which may probably be due to its nanoscale properties such as enhanced active surface area, good dipsersibility, good solubility, faster cell entry and enhanced bioavailability.

Conclusion

We have investigated the hepatoprotective effect of curcumin nanocrystals of 26 nm size. Our findings suggest that curcumin nanocrystal at a dose of 40 mg/kg body weight prevents oxidative stress by restoring the hepatic antioxidant and detoxification status in DMH treated rats. As oxidative stress and the antioxidant cum detoxification system imbalance are associated with many diseases, we could conclude that the curcumin nanocrystal at 40 mg dose may be of immense use in therapeutics. As a result, the product may occupy the forefront of the therapeutic compounds for treatment of human diseases.

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