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STUDY OF PROTECTIVE EFFECT OF CURCUMIN ON HEAT STRESS-INDUCED HEPATIC DAMAGE IN RATS

Pharmacology

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ABSTRACT

Background: Curcumin (CM) has been reported to protect liver against toxic injury. Recently, we reported hepatotoxic effects of moderate heat stress (HS) in male rats.

Aims & Objective: Present study incorporates CM protective effects against HS-induced rat hepatocellular damage.

Materials and Methods: CM (0.5 g/kg and 2.0 g/kg) was given orally as water suspension to rats for five consecutive days. Rats were subjected to HS daily one hour after CM administration. Assessment of status of liver enzymes, morphometric and morphological parameters were done both in unstressed (US) and HS and CM plus HS rats.

Results: HS Caused increased levels of liver enzymes (82.6 to 127.8%, P<0.001) and enhanced hepatic morphological and morphometric toxic paradigms compared to US rats. Treatments with CM in HS rats caused dose-dependent reversal of the enzyme levels (7.9 to 56.3%, P<0.1 to P<0.001) and improvement in liver morphology and morphometric parameters.

Conclusion: CM thus, indicated the hepatoprotective effects against HS- induced liver damage.

KEYWORDS

Heat stress-hepatotoxicity, Curcumin, Hepato-protection

Introduction

Curcumin longa (CL) has been reported to have therapeutic effects in various types of arthritis, Parkinsonism, Alzheimer's, cardiovascular, diabetes, and pulmonary diseases (1). Curcumin (CM), a major principal in CL was reported to have hepatoprotective effects of against CCl_4-, chloroquine- and thermally oxidized sunflower oil-induced oxidative damage presumably due to active role of antioxidants, cell proliferation and healing (2-4).

Moderate heat exposure (HS) in rats was reported to cause hepatic tissue damage and an increase in various liver enzymes which could be due to diversion of hepato-splanchnic vascular blood flow to cutaneous vasculature leading to hepatocellular hypoxia and damage (5).

The present study was undertaken to ascertain the hepatoprotective effects of CM against HS-induced hepatic damage in rats.

Materials and Methods

Animals — Inbred male Wistar strain rats (150 - 180 g) were used following approval by the Animal Ethical Committee of HIMS, Dehradun. Rats were housed singly in polypropylene cages (43cm X 29cm X 15cm) with a wire mesh top and a hygienic bed of rice husk under standard laboratory conditions of 25 ± 2°C (relative humidity 65 - 85%), with light and dark cycles of 10 and 14 h respectively. The animals were given water *ad libitum* and fed freshly cooked food[5]. "Principles of laboratory animal care" (NIH Publication No. 82-23, revised 1985) guidelines were followed (6).

Drug — CM powder was obtained (CUR-500™ capsule, >95% pure; Indsaff, Batala, India) and stored at normal room temperature, but away from direct sun light and moisture.

HS-induced hepatic damage — Animals were divided into four groups (n=6). Rats received equal volume of distilled water (DW)/CM suspension in DW (1 ml/100g) orally (7). CM water suspension was given daily at 10 am for 5 consecutive days and the last dose was given on 5th day one h before subjecting the rats to experiment. 1st and 2nd groups of rats received DW orally while; the 3rd and 4th groups received CM in the dose of 0.5 and 2.0 g/kg. Food was withdrawn daily in all the groups 4 h prior to heat exposure but water was allowed *ad libitum*. 1st group was not exposed to any heat but kept at room temperature of 25 ± 2°C (US, Negative control) while, 2nd (positive control), 3rd (CM, 0.5 g/kg) and 4th (CM, 2.0 g/kg) groups were exposed to moderately high environmental temperatures (HS, 37 ± 0.5°C) in a Biological Oxygen Demand (BOD) incubator (relative humidity 65-82%) for 4 h daily (11:00 am to 03:00 pm) for 5 consecutive days (5). On day 5 of experiment, the animals were sacrificed after US/HS with mixture of Ketamine (50 mg/Kg) and Xylazine (6.8 mg/kg) given by intra-peritoneal route. Blood was collected and liver removed in a petri-dish containing cold formalin (40%) for further study (5).

WBGT (Wet bulb globe temperature) index — It was calculated for five consecutive days of the heat exposure to the animals kept at room temperature and BOD incubator in the laboratory to assess the heat stress.

Biochemical observations — Serum SGPT, SGOT and ALKP were estimated by a semi-automatic RA-50 analyzer using the diagnostic reagent kit (DiaSys International).

Morphological observations — The liver pieces were fixed in 40% cold formalin-saline for 24-30 h, dehydrated in graded ethanol and finally embedded in paraffin. Three to five micrometer thick paraffin sections were cut, and stained with hematoxylin–eosin stain for examination under the light microscope.

Morphometric observations: The morphometric observations on the liver sections were done by the intersection-point counting method, using simple square lattice test system A 100 producing 40 X magnifications (5, 7).

i) Numerical density of hepatocytes (Nvh) — Number of hepatocytes in unit volume of liver was estimated from the numerical density of hepatocytes nuclei (Nvn), calculated from the relationship 1/D. Na/Pt. d2. k2 where Na is the number of profiles (hepatocytes nuclei) per unit area, D is the mean tangent diameter of hepatocytes nuclei,Pt is the test point number, d is the test line distance in the square lattice and k2 is a constant.

ii) Volume density of hepatocytes (Vvh) — It expresses the volume fraction of liver tissue occupied by the hepatocytes. It was calculated by the formula Pa/Pt where Pa is the number of test points found enclosed within profile of the hepatocytes, and Pt is the test point number.

iii) Numerical density of Kupffer cells (Nak) — It was expressed as the number of Kupffer cells per cm^2.

Acute toxicity study —Acute toxicity study was conducted with CM using graded doses of 0.5, 2, 5 and 10 g/kg, administered orally to 6

male albino rats in each group. Rats were observed continuously for first 6 h and then monitored for 72 h for any mortality or change in general behavior, signs of discomfort and nervous manifestations in the animals.

Data analysis: Data are expressed as mean ± SEM of 6 animals in each group. The data was analyzed by using both quantitative and qualitative techniques. Analysis of quantitative data was done by using the students' paired t-test, ANOVA and Tukey-Kramer Multiple Comparison Test. The program "GraphPad Instat 3" was used for this analysis.

Results

Acute toxicity study — No mortality or changes in general behavior, signs of discomfort and nervous manifestations were observed even with the highest dose of 10 g/kg.

Wet bulb globe temperature index (WBGT Index) — The calculated WBGT indexes for animal at room and BOD incubator were 24.4 ± 0.3°C and 37.0± 0.05°C (P <0.001). However, the general activity of animals exposed to heat was sluggish about 2 h compared to unexposed animals but the animals of all the four groups appeared to be healthy.

Biochemical Observations — HS caused increase in the serum levels of liver enzymes compared with US group (82.6 to 127.8% increase, P<0.001). Treatment with low dose CM (0.5g/kg) either tended to decrease or decreased their levels (7.9 to 23.5% decrease, P<0.1 to P<0.05) while higher dose (2.0 g/kg) caused significant decrease in their levels (44.1 to 56.3%, P<0.001) near to their basal levels (Table 1).

Table 01: Effect of low and high dose of curcumin (CM) treatments on moderate heat exposure (HS)-induced changes in liver enzymes

Oral Treatment (g/kg, once daily x 5 days)	**SGPT** (IU/L)	**SGOT** (IU/L)	**ALKP** (IU/L)
US DW	36.0 ± 1.16	102.0 ± 6.92	199.8 ± 11.6
HS DW	82.0 ± 5.67*	186.3 ± 6.19*	439.2 ± 12.5*
HS + CM 0.5	62.7 ± 4.12a	171.5± 5.48	390.2± 8.41a
HS + CM 2.0	35.8 ± 3.35c	104.2± 4.51c	198.3± 15.3c

Results are Mean ± SEM of 6 animals in each group.

P *<0.001 compared with US (heat unexposed negative control) group and P [a]<0.05 and [c]<0.001compared with HS (heat exposed positive control) group.

Morphological Observations

The morphological findings in the US and HS rat liver are depicted in Fig. 1 and 2 respectively. Histology of liver of HS rats showed cell swelling, ballooning degeneration, single cell necrosis along with small foci of necrosis disrupting cell plates in lobules and compression of sinusoids. These changes were pronounced in periportal and mid-zonal areas. Kupffer cell hyperplasia was observed in all the cases. At places, hepatocytes show regenerative changes as binucleate cells and anisocytosis.

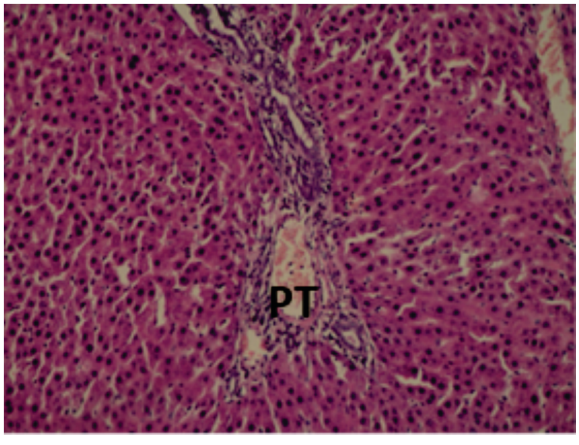

Figure 1:.US rat showing single cell thick liver cell plate, normal sinusoids, and portal triad (PT).Mild to moderate mononuclear infiltration in portal area (400X).

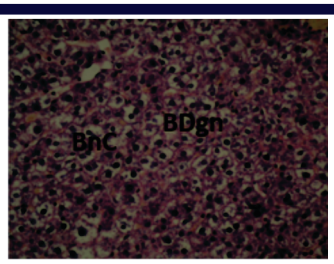

Figure 2: HS rat showing foci of necrosis mainly in zone 2, disruption of liver cell plate, ballooning degeneration (Bdgn), binucleate cells (BnC), and Kupffer cell hyperplasia (400X).

When compared with HS-induced damage, low dose of CM showed little histological improvement in cell swelling and ballooning degeneration, Kupffer cell hyperplasia, binucleate cells and anisocytosis (Figure 3). Higher dose of CM on the other hand, showed marked histological improvement as indicated by occasional single cell necrosis, mild swelling of hepatocytes and maintenance of single cell thick liver cell plates along with sinusoidal dilatation (Figure 4). The architecture of the liver treated with higher dose of CM was similar to those present in control US group.

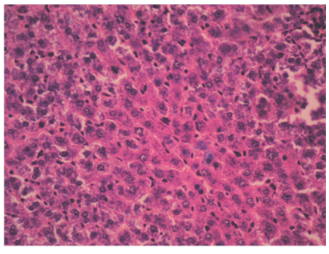

Figure 3: liver of low dose CM (0.5 mg/kg) + HS showing decreased cell swelling and Kupffer cell hyperplasia (400X).

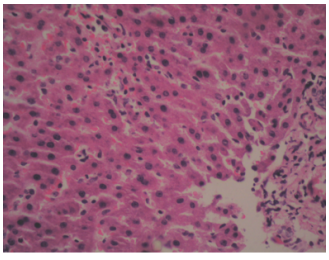

Figure 4: liver of CM (2.0 mg/kg)+HS showing sinusoidal dilatation. Liver cell plates were maintained and single cell thick. Kupffer cell hyperplasia and necrotic foci were not seen (400X).

Morphometric Observations

HS showed increase in all the morphometric parameters viz. Vvh, Nvh, and Nak (55.9 to 72.6 % increase, P<0.001) when compared with US group. Again treatments with low and high doses of CM caused a dose-depend decrease (6.3 to 30.7% decrease, P<0.05 to P<0.001) in all the parameters indicating the reversal in morphometric parameters and reduction in hepatic damage, more marked at high dose (Table 2).

Table 02: Effect of low and higher dose of CM treatments on HS-induced changes in morphometric parameters of liver

ORAL TREATMENT (g/kg, once daily x 5 days)	Nvh (millions/mL)	Vvh (%)	Nak (per cm2)
US DW	14.9± 0.24	0.79± 0.017	4914± 51.6
HS DW	19.0± 0.38*	0.93± 0.014*	7081± 74.9*
HS + CM 0.5	17.8 ± 0.40a	0.87 ± 0.021a	6855 ± 58.4a
HS + CM 2.0	16.9± 0.39c	0.80± 0.016c	5408± 91.6c

Results are Mean ± SEM of 6 animals in each group.

P *<0.001 compared with US group and P [a]<0.05; [c]<0.001 compared with HS group.

DISCUSSION

WBGT index is an indicator for heat-stress[9] and increased when the animals are exposed even to moderately high environmental

temperatures of 37.0 ± 0.5°C from the ambient temperatures of 24.4 ± 3°C. The above index was maintained to induce HS to animals.

SGPT, SGOT, and ALKP are considered to be the most sensitive markers of liver damage because they are cytoplasmic in location and are released into the circulation after hepatocellular damage (3). HS caused liver injury as indicated by an increase in serum levels of liver enzymes. The above changes produced by HS were reversed dose-dependently with the treatments by CM. CM was also reported to show protective effects against various others hepatotoxic substances in terms of enhanced enzymatic levels affected during liver damage (1). The protective effects of CM as reported earlier against toxic stimuli could be due to its antioxidant effects causing scavenging of free radicals, interacting with oxidative cascade and preventing its outcome, quenching oxygen and making it less available for oxidative damage as reported earlier (4).

Histological and morphometric studies indicated marked hepatic cell swelling, ballooning degeneration, single cell necrosis along with small foci of necrosis disrupting cell plates in lobules, and sinusoidal compression in HS, a direct evidences of hepatocellular damage which was similar to the findings as observed against HS-, CCl_4- and chloroquine-induced liver injury (2,3,5,10). The dose-dependent protective effects observed with CM (0.5-2.0 mg/kg), against HS, showed a minimal histological improvement in low dose while, high dose of CM, showed remarkable histological improvement as noticed with the maintenance of hepatic architecture near to US animals indicating good hepato-protection.

Nak, a parameter of hyperplasia of Kupffer cells, was found to increase by HS-induced damage to liver cells indicating Kupffer cell hyperplasia and these changes were also observed in other studies (5, 10, 11). The morphometric parameters like Vvh and Nvh were raised in HS rat liver indicating a continued proliferation of hepatocytes and healing which confirm the regenerative power of the liver. These changes are very similar to changes observed in earlier studies (5, 8, 10).

The most probable cause of these injuries could be oxidative stress as evidenced by dose- dependent protective effects of CM, the low dose producing less effect than the higher dose. Many herbal plants extracts/active principles including CM have been reported to have good antioxidant effects (12, 13). Pro-inflammatory markers also play important effects on healing and toxic substances causing liver damage have been reported to enhance them (14). CM has been reported to decrease the inflammatory markers paving the way for reduction in tissue damage (15). Further, studies regarding the status of oxidative damage (free radicals versus antioxidants) and inflammatory markers like cytokines, TNFα, IL1β and C-reactive proteins affected in HS should be carried out to come to definite conclusions regarding their role in HS-induced cytotoxicity and their decrease leading to protective effects of CM.

Conclusion:

Curcumin was thus, found to have hepatoprotective effects as observed on morphological, morphometric, and biochemical parameters of liver damage functions against repetitive heat stress of moderate level in adult Male Wistar albino rats.

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