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Antioxidant Potential, Anti-Inflammatory and Hepatoprotective Effect of Curcumin in a Rat Model of Hepatotoxicity

Samy A. Hussein*; Omaima A. Ragab; Yakout A. El Senosi; and Shaimaa A. Abdel-Muttalib

Department of Biochemistry, Faculty of Vet. Med., Benha University, Egypt.

*Corresponding author: Samy Ali Hussein; email: samyaziza@yahoo.com

ABSTRACT

Curcumin [1,7- bis (4-hydroxy-3-methoxyphenyl)- 1,6- heptadiene- 3,5-dione] was shown to exert potent antioxidant, anti-inflammatory and hepatoprotective properties. This study was done to investigate the protective effects of curcumin in thioacetamide (TAA)-induced hepatic injury in rats. Thirty-six male albino rats were divided into three equal groups. Group I (normal control group). Group II (TAA-intoxicated group): rats received thioacetamide (50 mg/kg b. wt. ip) twice weekly for 6 weeks. Group III (TAA + curcumin co-treated group): rats received thioacetamide (50 mg/kg b. wt.) and simultaneously administered curcumin (200 mg/kg b. wt./daily/orally) for 6 weeks (end of experiment). All animals were sacrificed after 6 weeks. TAA administration induced liver damage manifested by the significant increases in serum levels of ALT, AST, ALP and total bilirubin compared with control group. Oxidative stress in the group II was manifested by a significant rise in L-MDA levels with a marked reduction in the activities of antioxidant enzymes like GST, catalase CAT and depletion of GSH content in liver tissues as compared with the control group. On the other hand, TAA significantly affected the inflammation markers represented by elevation of myeloperoxidase (MPO) activities and upregulation of interleukin-6 (IL-6) gene expression levels in liver tissues. The coadministration of curcumin and thioacetamide (protection modality) prevented liver injury by normalizing the biochemical parameters, lipid peroxidation index and improving the protein oxidation, inflammatory markers and the antioxidant status. These findings suggested that, liver injury could be curtailed by the antioxidant and anti-inflammatory activities of curcumin and the normal status of the liver could be preserved.

Keywords: Thioacetamide, curcumin, oxidative stress, inflammation, hepatoprotective, rats.

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1. INTRODUCTION:

Liver is a pivotal organ for metabolism and detoxification (Bader and Mir, 2017). Thus liver diseases remain one of the serious and worldwi de health problems which are associated with significant morbidity and mortality (Alshawsh et al., 2011). Drug induced liver injury is one of the most common causative factor of liver diseases, that poses a major clinical and regulatory

challenge (certain antibiotics, chemotherapeutic agents, high doses of paracetamol, carbon tetrachloride (CCL4), thioacetamide (TAA), etc.) ((Russmann et al., 2009); Alshawsh et al., 2011).

Appropriate animal models have contributed to our understanding of the mechanisms responsible for hepatotoxic injury. The pathological lesions caused by hepatotoxins may be similar to many forms of liver disease, contributing to the evaluation of novel potential hepatoprotectants (Abul et al., 2002);(Bruck et al., 2004). Thioacetamide (TAA) is a potent selective hepatotoxin used in several experimental models (Aydin et al., 2010). It has been used to induce a model of acute and chronic liver injury in rats (Ahmed et al., 2012, Gupta and Dixit, 2009) . The toxic effects of thioacetamide (TAA) have been attributed to the metabolic products that result from its bioactivation. TAA undergoes a two-step bioactivation that is mediated by the microsomal cytochrome P450 isozyme (CYP2E1) to thioacetamide sulphoxide as well as, to a reactive metabolite, thioacetamide-S, S-dioxide (Chilakapati et al., 2005). The latter binds to tissue macromolecules and is responsible for the change in cell permeability, increased intracellular concentration of Ca^{2+}, increase in nuclear volume and enlargement of nucleoli and inhibits mitochondrial activity eventually leading to hepatic necrosis (Bautista et al., 2010). Thioacetamide (TAA) causes centrilobular necrosis, which has been shown to induce reactive oxygen species (ROS) production, lipid peroxidation, periportal inflammatory cell infiltration and apoptosis in rat liver (de David et al., 2011). Therefore, biotransformation of TAA precedes oxidative damage associated liver injury. Nevertheless, the detailed biochemical mechanisms underlying this hepatotoxic process of TAA remain largely unknown (Ahmed et al., 2012).

Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects (Patil and Mall, 2012). The use of natural remedies and nutritional compounds for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or another for this purpose (Anbarasu et al., 2012).

Curcumin or diferuloylmethane (1,7-bis[4-hydroxy-3-127methoxyphenyl]- 1,6 heptadiene- 3,5-dione) is a hydrophobic poly-128 phenol compound naturally concentrated in the rhizome of the 129 herb Curcuma longa, commonly known as turmeric (Altenburg et al., 2011).

It has been shown to exhibit several activities including antioxidant (Al-Jassabi et al., 2012), antimicrobial (Tajbakhsh et al., 2008), anti-inflammatory (Bereswill et al., 2010), antiviral (Kutluay et al., 2008) , anti-carcinogenic (Huang et al., 2013) and anti-hepatotoxic properties (García-Niño and Pedraza-Chaverrí, 2014).

The present study investigated the prospective protection and anti-inflammatory effect of curcumin against hepatotoxicity induced by thioacetamide in male rats.

2. MATERIALS AND METHODS:

2.1. Experimental animals:

thirty-six adult white male albino rats of 6-8 weeks old and weighing 150 – 200 g were used in this study. They were obtained from the breeding unit of Egyptian Organization for Biological Products and Vaccines (Abbassia, Cairo). Rats were housed in separated in steel mesh cages and kept under conventional laboratory conditions throughout the period of

experiment. The rats were fed a standard rat pellet diet and fresh, clean drinking water was supplied ad-libitum. Animals were maintained under standard conditions of ventilation, temperature (25±2°C), humidity (60-70%) and light/dark condition (12/12h). All rats were acclimatized for a period of 15 days prior to the beginning of study. The local committee approved the design of the experiments, and the protocol conforms the guidelines of the National Institute of Health (NIH) and National Research Center- Medical Research Ethics Committee for the use of animal subjects.

2.2. Chemicals and antioxidant:

All chemicals were of analytical grade and obtained from standard commercial suppliers. The antioxidant and chemicals used in the present study were:

a. Thioacetamide (TAA) extra pure was purchased from (Merck, Darmstadt Germany). TAA was prepared freshly by dissolving in sterile distilled water. The resultant suspension was administered ip to animals twice a week at a doses of 50 mg/kg b.wt. (Anbarasu et al., 2012).

b. Curcumin was purchased from Sigma-Aldrich Company, Los Angeles, USA. it was dissolved in 7%DMSO freshly prepared and administered orally to rats at a dose level of (200 mg/kg b. wt.) once daily (Shapiro et al., 2006).

c. Other chemicals used in this study were of the highest purified grades available purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt and Bio-diagnostic Co., Cairo. Egypt.

2.3. Experimental design:

After acclimatization to the laboratory conditions, the animals were randomly divided into three groups (12 rats each) placed in individual cages and classified as follow:

Group I (normal control group): Rats received no drugs, served as control non- treated for all experimental groups.

Group II (thioacetamide intoxicated group): Rats were weighted and received thioacetamide at a dose level of (50 mg/kg b. wt.) intraperitoneally twice weekly for 6 weeks.

Group III (thioacetamide + curcumin co-treated group): Rats were administrated curcumin (200 mg/kg b. wt./ orally, daily) and simultaneously administered thioacetamide (50 mg/kg b. wt.) intraperitoneally twice weekly 2h after the respective assigned treatment for 6 weeks.

2.4. Sampling:

2.4.1. Blood samples:

Twenty-four hours fasting after the last dose of the drugs treatment administration, rats were anaesthetized under diethyl ether anesthesia. Blood samples were collected by ocular vein puncture in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minutes. The serum was taken by automatic pipettes and collected in dry sterile tubes, then kept in deep freeze at -20 °C until use for assay of the liver biomarker. All sera were analyzed for determination of the following parameters: AST, ALT, ALP and total bilirubin.

2.4.2. Tissue samples:

The rats were then sacrificed by cervical dislocation and the livers were immediately harvested, washed several times in normal saline, blotted between two damp filter papers, weighed and stored at -80°C for

subsequent biochemical analyses.

2.4.2.1. Liver tissue for biochemical analysis

Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volumes of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for the determination of the following parameters: L-MDA, MPO, CAT and GST activities.

0.2 g of liver tissues were 0.2 g of liver tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.: 253-433-4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed and incubated for 1 hour and centrifuged for 10 min at 3,000 r.p.m then the clean supernatant was removed and used for determination of GSH concentration.

2.4.2.2. Liver tissue for molecular gene expression (anti-inflammatory parameter)

About 0.5 g of liver tissue put in eppendorf tubes and were immediately kept in liquid nitrogen and stored at -80°C till RNA extraction for determination of IL-6 level.

2.5. Biochemical analysis

Serum ALT, AST were determined according to the method described by Schumann et al., (2002) and serum ALP activity was determined enzymatically according to EL-Aaser and EL-Merzabani, (1975). Liver tissue L-MDA, MPO, CAT and GSH were determined according to the method described by Mesbah et al., (2004), Bradley et al., (1982), Kakkar et al., (1984), Xu et al., (1997) and Patterson and Lazarow, (1955) respectively. Moreover, the mRNA expression level of IL-6 was determined by real-time quantitative polymerase chain reaction (real- time qPCR) analysis in liver of rats. Target gene was normalized with β – actin by used the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.6. Statistical analysis:

The results were expressed as mean ± SE using SPSS software program version 16 (SPSS© Inc., USA). The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparison among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p<0.05$.

3. RESULTS:

The data shown in table (1) illustrated that, serum ALT, AST and ALP activities also total bilirubin conc. were significantly elevated ($P\leq0.05$) in rats received TAA alone when compared with the control group. Curcumin (200 mg/kg b.wt.) treatment to thioacetamide intoxicated male rats significantly prevented these changes, resulting in a remarkable protection ($P\leq0.05$) regarding the same parameters when compared with thioacetamide exposed group. With the ability to restore ALT, AST and total bilirubin levels to the normal level of control group.

The data summarized in table (2) revealed that, thioacetamide intoxicated rats showed significant induction in liver tissue L-MDA, MPO and significant up-regulation of IL-6 when compared to normal control group ($P\leq0.05$). Administration of curcumin induce noticeable change in TAA intoxicated male rats causing a significant decrease in elevated liver tissue L-MDA, MPO and a significant

down-regulation IL-6 gene expression when compared with TAA toxic group.

In the experimental group that received TAA alone, there was a significant reduction in hepatic CAT, GST activities as well as reduced glutathione content GSH (P≤0.05) compared with control rats. However, in the group that received TAA and curcumin, these hepatic antioxidant parameters were significantly elevated in comparison with the TAA group, returning to levels almost near to controls (Table 3).

Table (1): Effect of curcumin administration on serum ALT, AST, ALP and total bilirubin activities in thioacetamide intoxicated male rats.

Parameters / Exp. groups	ALT (U/L)	AST(U/L)	ALP(U/L)	T. bilirubin (mg/dl).
Group I: Normal control	46±6.08^{c}	187.66+10.78 ^{b}	429.33±25.42 ^{c}	0.176+.047 ^{b}
Group Π: Thioacetamide (TAA)group	128.33±4.04^{a}	450.66+16.65 ^{a}	1133.66±48.54 ^{a}	1.10+.240 ^{a}
Group III: TAA+ curcumin	55±7.93^{bc}	193+13.22 ^{b}	498.66±14.04 ^{b}	0.220+.070 ^{b}

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table (2): Effect of curcumin administration on liver tissue L-MDA, MPO and IL-6 levels in thioacetamide intoxicated male rats.

Parameters / Exp. Groups	L-MDA (nmol/g tissue)	MPO (µ/mg of tissue)	Fold change in IL-6 gene expression
Group I: Normal control	0.773±.047 ^{c}	0.05 ± 0.002 ^{c}	1.00± 0.04^{e}
Group Π: Thioacetamide (TAA)group	1.59±.153 ^{a}	0.61±0.01 ^{a}	10.48± 0.32^{a}
Group III: TAA+ curcumin	1.28±.052 ^{b}	0.29±0.008 ^{b}	3.68 ±0.12^{c}

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table (3): Effect of curcumin administration on liver tissue GST, CAT activities and GSH concentration in thioacetamide intoxicated male rats.

Parameters / Exp. Groups	GSH µmol	CAT (µ/g tissue)	GST (µ/g tissue)
Group I: Normal control	7.74+.769 [a]	1.14+.098 [a]	87.88+.690 [a]
Group II: Thioacetamide (TAA)group	4.40+.140 [c]	.830+.098 [b]	63.97+5.84 [b]
Group III: TAA + curcumin	5.94+.540 [b]	1.05+.060 a	90.97+5.74 [a]

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

4. DISCUSSION:

The present study evaluated the effects of curcumin treatment on liver injury resulting from TAA intoxication. Administration of thioacetamide (50 mg/kg b. wt., twice weekly ,6 weeks) significantly elevated the serum transaminases, ALP and total bilirubin activities when compared to the normal rats. This result was in agreement with previous data by Esmat et al., (2013) who reported that, TAA intoxication caused dramatic increases in serum conjugated bilirubin (112.5%), ALT (168.53%), AST (493.91%), and ALP (272.62%) activities and the AST/ALT ratio (140.43%) compared with normal control rats.

The increase in the activities of AST, ALT, ALP and total bilirubin in serum of rats treated with TAA might be due to the increased permeability of plasma membrane or cellular necrosis leading to leakage of the enzymes to the blood stream (Al-Attar, 2011). The stimulation of hepatic regeneration was known to make the liver more resistant to damage by toxins. ALP is excreted normally via bile by the liver. In the liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in the increased activity of serum ALP (Singh et al., 1998).

Meanwhile, there was a significant ($P \leq 0.05$) restoration of these enzyme levels on administration of curcumin at a dose of (200 mg/kg b. wt.). These findings are in harmony with results of Ali et al., (2016) who indicated significant decreases in serum aminotransferases, and total and direct bilirubin levels in rats group given curcumin when compared to the untreated TAA intoxicated group. In another study , a significantly lower level was observed for the liver-specific enzymes(AST, ALT and ALP) in rats group treated with TAA and curcumin compared with the TAA-treated group (Fazal et al., 2014). Serum levels of transaminases return to normal may be a consequence of healing of hepatic parenchyma, stabilization of plasma membrane regeneration of hepatocytes, as well as repair of hepatic tissue damage caused by TAA (Palanivel, 2008, Vadivu et al., 2010, Moustafa et al., 2014). The suppression of serum ALP activity in the

rats treated with TAA and curcumin suggests stabilizing hepatobiliary dysfunction of the rat liver during chronic injury with TAA (Moustafa et al., 2014) .In general, the marked decrease in serum transaminases, ALP and total bilirubin demonstrates the preventive and curative effect of curcumin in TAA intoxication.

Significant higher levels of MDA (indicator of lipid peroxidation) have been observed in rats exposed to TAA. In several studies, TAA administration resulted in a noticeable increase in liver MDA levels (Aydin et al., 2010, Alshawsh et al., 2011). TAA-induced lipid peroxidation is reported to disturb the reliability of the membrane, leading to inhibition of membrane-bound enzymes.

The boost in lipid peroxidation and generation of ROS may diminish cell viability (Fazal et al., 2014). A significant decline in lipid peroxidation was found after the treatment with curcumin; therefore, it can be said that the curcumin restrained the production of ROS, which has been shown to decrease lipid peroxidation possibly by its antioxidant mechanism (Ali et al., 2014).

Higher levels of production of H_2O_2 lowered the activity of CAT (Stief, 2003). The activity of CAT was found to be significantly lower ($p<0.005$) in TAA-treated rats as compared with the control group, and after treatment with curcumin, CAT activity was found to be significantly reducing the effects of TAA in liver (Al-Jassabi et al., 2012).

The non-enzymatic anti-oxidant GSH is thought to be a vital player in the process of detoxification, which energetically participates in reactions which lead to the obliteration of H_2O_2, free radicals, and other foreign compounds (Rana et al., 2002, Kamanli et al., 2004). The heavy depletion of GSH to scavenge the toxic intermediates formed by TAA led to considerably lower levels of GSH content in liver tissue of the TAA group. On the other hand, a significant rise in GSH level was observed in the case of curcumin administration to TAA-induced liver toxicity. This result agrees with García-Niño and Pedraza-Chaverrí (2014) who reported a preventive effect of curcumin on glutathione (GSH) depletion induced by heavy metals.

The GST superfamily comprises enzymes that protect healthy cells from reactive oxygen species (ROS), exogenous toxins, and cytotoxic agents. Inactivation of GST can create a deficit of detoxification capacity and increase tissue susceptibility towards carcinogens and ROS (Maruyama et al., 2002). The present study indicated a significant reduction in hepatic GST activity when compared with normal control group($p<0.05$). These findings concur with that of Spira and Raw, (2000) who supposed that GST activity inhibition is due to the deleterious effect of TAA on GST transcription. Treatments of curcumin (200mg/kg) markedly restored the TAA induced decrease in hepatic GST activity at ($p<0.05$). This result was supported by Dubey and Owusu-Apenten, (2014) study.

MPO is an enzymatic constituent of Kupffer cells. Primary injury from the toxic metabolites of TAA activate the Kupffer cells in liver, leading to increased activity of MPO and the release of pro-inflammatory cytokines such as IL-6 and TNF-α. (Andrés et al., 2003). The obtained results revealed a significant ($P\leq0.05$) induction of myeloperoxidase (MPO) activity in livers of TAA-intoxicated rats. Similarly, Yogalakshmi et al., (2010)

reported a significant increase in MPO activity after TAA intoxication. Administration of curcumin significantly reduced TAA-induced activity of the MPO in hepatic tissues (Sandersen et al., 2015).

Although the exact molecular mechanisms by which TAA induces toxic effects in different organs is not understood, it is well known that TAA interferes with ribosomal activity, by this means stimulating DNA synthesis and interfering with protein synthesis (Al-Bader et al., 1999).

IL-6 gene expression was significantly raised in the TAA group as compared with the control group, indicating that TAA induced changes in the IL-6 gene, producing more IL-6 protein than would the normal gene. This result comes in concomitant with recent literature which illustrates that in TAA-induced acute hepatic lesions, INF-γ, TNF-α and IL-6 for M1-factors and IL-4 for M2-factors are already increased before histopathological change (Yamate et al., 2016).This increase in IL-6 gene expression was overcome in the experimental animals who received curcumin with TAA; hence, they seemed to be protected against abnormally increased levels of IL-6 in the liver caused by TAA. This result was confirmed by several previous studies (Bereswill et al., 2010, Tarladacalisir et al., 2013).

5. CONCLUSION:

We concluded that curcumin co-treatment significantly curtails the toxic effects of TAA in liver. This attenuation is verified by remarkable anti-hepatotoxic effect (reduction in the activities of ALT, AST and total bilirubin), antioxidant (induce expression and activities of CAT, GSH and GST) (reduce Lipid oxidation) and anti- inflammatory effect (suppress MPO and IL-6). These abilities of curcumin make it a potential drug for preventing liver damage induced by hepatotoxin.

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