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Protective Effect of Curcumin versus N-acetylcystein on Acetaminophen Induced Hepatotoxicity in Adult Albino Rats

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Abstract

Curcumin exerted hepatoprotective influences on various animal models of liver injury such as carbon tetrachloride, endotoxin and thioacetamide. This Study aimed to investigate the protective effects of curcumin as compared to N-acetylcysteine in the rat model of acetaminophen induced hepatotoxicity. 55 albino rats were divided into four groups: group (control group). Group II received acetaminophen. Group III received both acetaminophen and N-acetylcysteine and Group IV received both acetaminophen and curcumin. At the end of the experiment, liver specimens were processed for histological study by light microscope and stained immunohistochemically for detection of apoptosis in hepatic cells by using anti p53 and the oxidative damage by anti-inducible nitric oxide synthase (anti iNOS). Serum levels of liver injury markers were assessed as well as the levels of malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) activity were determined in all dissected tissues. Histological examination of liver sections of acetaminophen treated group revealed degeneration, cytoplasmic vacuolation and hydropic necrosis of hepatocytes. The central and the portal veins were dilated and congested and invading infiltrative inflammatory cells were appeared in association with significant increase in p53 and iNOS positive cells. Significant rise in serum levels of liver injury markers and MDA in liver tissues were recorded. However, levels of GSH and SOD were significantly decreased. Both curcumin and N-acetylcysteine resolved most of these morphological, immunohistochemical and biochemical alterations. Curcumin has protective effect similar to N-acetylcysteine against liver damage induced by acetaminophen in rats by reducing oxidative stress and apoptosis.

Keywords: Acetaminophen; Albino Rats; Curcumin; Hepatotoxicity; N-acetylcystein

Introduction

Acetaminophen (paracetamol, N-acetyl p-aminophenol; APAP) popularly known as paracetamol, is randomly and widely used everywhere in the world as a common analgesic and antipyretic drug. It is the most commonly reported cause of acute liver failure in the western world [1]. The main safety concern with using paracetamol is to avoid over dosage; the intake of an overdose of acetaminophen frequently causes severe acute liver injury [2]. An acute acetaminophen overdose may give rise to potentially fatal hepatic and renal necrosis in humans [2] and experimental animals [3].

Acetaminophen overdose causes liver injury by mechanisms involving glutathione depletion and oxidative stress. Previous studies showed that APAP binds to cellular proteins including mitochondrial membrane directly and causes mitochondrial dysfunction, induction of ROS and necrotic cell death [4]. The role of apoptosis in acetaminophen-induced cell killing is still unconfirmed. Some studies revealed that acetaminophen produced DNA fragmentation, which suppose apoptotic cell death, in hepatocytes [5]. The protein P53 is a nuclear phosphoprotein that directs the transcription of DNA, cellular proliferation and cellular apoptosis. Mutations accumulation of P53 gives rise to modified proteins that can be detected by immunohistochemistry [6].

In recent years, natural products from plants have received considerable attention as a rich resource for drug development. Curcumin (CMN), a phenolic phytochemical responsible for the yellow color of turmeric (Curcuma longa), has been identified to be a powerful anti-inflammatory, anti-cancer and antioxidant agent, and is under preclinical trial for cancer prevention [7]. It also has potential therapeutic effects against neurodegenerative, metabolic, pulmonary, cardiovascular and autoimmune diseases [8]. Additionally, Curcumin exerted hepatoprotective influences on various animal models of liver injury such as carbon tetrachloride [9], endotoxin [10] and thioacetamide [11]. There is a proof that curcumin improves liver detoxification by increasing the activity of glutathione-S transferase, an enzyme which conjugates glutathione with a wide categories of toxins to enables their removal from the body [12].

N-acetylcysteine (NAC) a sulfhydryl substance is a derivative of amino acid L-cysteine. It is a precursor of reduced glutathione and has direct antioxidant activities [13]. Detoxifying effects of NAC were lighted on in the 1970s and since then NAC was being used as an antidote to acetaminophen intoxication and it is the traditional therapy for treatment of the APAP overdose patient [14,15].

N-acetylcysteine acts as an antioxidant exhibiting direct and indirect activities [16]. It re-establishes the intracellular GSH, which is diminished during oxidative stress and inflammation

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[17]. N-acetylcysteine has been shown to do its antioxidant effect by scavenging the free radicals such as OH (hydroxyl radical), H_2O_2 (hydrogen peroxide) and O_2 (superoxide radical) [18]. As a source of sulfhydryl group it consequentially promotes reduced glutathione biosynthesis [19].

Most of published data focused mainly on serum biochemical alterations induced by curcumin on paracetamol overdose without a precise description about the changes in histological structure and immunohistochemical expression of apoptotic or oxidative markers occurred during hepatic toxicity. Therefore, This study was planned to investigate the protective effects of curcumin as compared to N-acetylcysteine in the rat model of acetaminophen induced hepatotoxicity based on biochemical, histological and immunohistochemical studies.

Materials and Methods

Animals

The present study was carried out on 55 pathogen-free adult male albino Wistar rats weighing 150-200 g. The rats were housed in polypropylene cages at room temperature (25 ± 3°C) with 12/12 hours light and dark cycle and the animals were fed with a balanced diet and tap water ad libitum. They were acclimatized to their environment at least 2 weeks before starting the experiment. All animal procedures were in accordance with the recommendations of the Canadian Committee for Care and Use of Animals [20] and were approved by the local Institutional Animal Ethical Committee. All experimental procedures were performed from 8 to 10 a.m.

Chemicals

Acetaminophen, N-acetylcysteine and curcumin were used in the present study and were purchased from Sigma Co. (St Louis, Missouri, USA).

Acetaminophen and N-acetylcysteine were prepared freshly by dissolving in normal saline. Curcumin was dissolved in olive oil.

Study protocol

The rats were divided into four main groups:

Group I: (the control group): This group included 25 rats that were further subdivided into five equal subgroups comprising five rats each:

Subgroup (i): The rats in this subgroup were kept without treatment throughout the whole period of the experiment.

Subgroup (ii): The rats in this subgroup received the diluting vehicle for acetaminophen and N-acetylcysteine (normal saline) orally daily by intragastric tube for 10 days.

Subgroup (iii): The rats in this subgroup received the diluting vehicle for curcumin in the form of 1 ml of olive oil once daily for 10 days through an intragastric tube.

Subgroup (iv): The rats in this subgroup received 100 mg/kg/day curcumin through gastric intubation once daily for 10 days [21].

Subgroup (v): The rats in this subgroup received 200 mg/kg N-acetylcysteine once daily through gastric intubation for 10 days [22].

Group II: included 10 rats that received 500 mg/kg acetaminophen dissolved in normal saline by gastric intubation as single oral dose for the last 5 days of the experimental period preceded by the diluting vehicle for acetaminophen orally daily by intragastric tube for the first 5 days [23,24].

Group III: 10 rats were treated with 200 mg/kg of N-acetylcysteine dissolved in normal saline daily by gastric intubation alone for five days then received both 500 mg/kg acetaminophen and 200 mg/kg of N-acetylcysteine for other 5 days.

Group IV: 10 rats were received 100 mg/kg of curcumin dissolved in olive oil daily by gastric intubation alone for five days then received both 500 mg/kg acetaminophen and 100 mg/kg of curcumin for other 5 days.

On 10th day of drug administration, the rats were anesthetized by xylazine 2% and Ketamine 10%; then 5 mL from their blood were taken from the heart. Afterward, the abdomen was opened, and the livers were removed and cleaned. Liver tissue samples were taken from the right lobe.

Histological and immunohistochemical examination

Parts of liver tissues were obtained from each animal and were fixed in 10% neutral buffered formalin for 24 h dehydrated in ascending ethanol series, cleared in xylene and embedded in paraffin wax. Sections of four micron-thickness were taken, stained with hematoxylin and eosin (H&E), and examined under a light microscope, to detect degeneration, vacuolization and necrosis [25].

The liver tissue sections were independently examined and scored by one of the authors; while the examiner was unaware of the group to which the specimen belonged. The degree of necrosis was expressed as the mean of 10 high power fields (HPFs), chosen at random and classified on a scale of 0 - 5 (no hepatocyte necrosis = 0; necrosis in few hepatocytes = 1; necrosis in more than 10% but less than 24% of hepatocytes, 2; necrosis in more than 25% but less than 39% of hepatocytes = 3; necrosis in more than 40% but less than 49% of hepatocytes = 4; and necrosis in more than 50% of hepatocytes = 5) as described and used by Silva [26].

For immunohistochemical study p53 was used for detection of apoptosis in hepatic cells, and anti inducible nitric oxide synthetase enzyme (anti iNOS) to detect oxidative damage. Paraffin sections of the liver were cut at 4 µ thicknesses on positively charged slides. Sections were deparaffinized in xylene, hydrated in descending grades of alcohol, and incubated in 3% hydrogen peroxide solution in methanol for 10 min to block endogenous peroxidase activity. These steps were followed by washing the sections in PBS and subsequently incubated 10 min at room temperature with 10% goat serum to block unspecific binding. The sections were then incubated overnight at 4 °C with the specific primary antibody for p53 and iNOS diluted in PBS. Thereafter, the sections were washed twice in PBS for 5 min each. Secondary antibodies were applied and the sections were again incubated for 20 min, followed by washing three times in PBS for 5 min each. Diaminobenzidine tetrahydrochloride solution was applied to the sections and they were further incubated for 10 min. The sections were washed in distilled water and counterstained with Mayer's hematoxylin (2 min) [27]. Negative controls were processed following the same protocol, but without the use of the primary antibody. Positive control sections for p53 were from human breast adenocarcinoma and for anti iNOS were from lung tissue.

Biochemical study

Assessment of serum levels of liver injury markers: the collected blood samples from all animals via cardiac puncture were allowed to clot. Their serum was removed by centrifugation at 1500 rpm for 10

min at room temperature. Serum samples were stored in a freezer (–20ºC). All serum samples were sterile and haemolysis-free. They were processed to determine the enzymatic activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) with a spectrophotometric technique by the Olympus AU-2700 auto analyzer and presented as IU/L.

Measurement of some oxidative stress factors: liver tissues from both control and treated rats were minced and homogenized (10%, w/v) separately in ice-cold saline, sucrose buffer (0.25 M sucrose, 1 mM EDTA and 0.05 M Tris–HCl, pH 7.4) in a Thomas Sci. Co. glass-type homogenizer (Teflon pestle). Homogenates were centrifuged at 18,000×g (+4 ◦C) for 15 min to determine oxidative stress factors:

The thiobarbituric acid substrate assay was used to measure malondialdehyde (MDA; nmol/g wet tissue) as an indicator of lipid peroxidation, with a spectrophotometer (at 535 and 520 nm) [28]. Ellman's method was used to measure reduced GSH (µmol/g wet tissue) as a biomarker of protective oxidative injury, with a spectrophotometer [29]. The xanthine/xanthine oxidase (XO) assay was used to estimate superoxide dismutase (SOD) activity (antioxidant) (Units/mg protein) by measuring the amount of reduced nitroblue tetrazolium (NBT), with one unit of SOD defined as the amount of protein that inhibits the rate of NBT reduction by 50% [28].

Quantitative morphometric measurement and statistical analysis

Leica Qwin 500 C Image analyzer computer system (Leica Imaging System LTD., Cambridge, England) in (Central Research Lab, Tanta Faculty of Medicine, Egypt) was used to obtain the morphometric data in this study. Ten non overlapping fields in slides of each animal of each group were examined to:

1- Quantifying the number of cells positive for the protein p53 staining at a 1000x magnification and were expressed as cell number per field.

2- The mean of optical density and area percentage of iNOS reaction at magnification of 1000 x. They were measured using the color detect menu and in relation to a standard measuring frame.

SPSS 16.0 statistical package was used to perform all statistical analyses. Comparisons between two groups were analyzed by unpaired Student 't' test (2-sided). However, the difference among more than two groups (control subgroups) was assessed with one-way analysis of variance (ANOVA) followed by Post Hoc Tukey's test. The results were expressed as mean ± standard deviation (SD). A probability (*P*) level of < 0.05 was considered statistically significant.

Results

Histological and immunohistochemical results

Hematoxylin and Eosin (H&E) stain: Histological results of liver specimens' examination of H&E stained sections of all control subgroups showed the characteristic hepatic architecture. Normal structure of the hepatocytes and central vein were observed in the liver section of control rats. The hepatocytes were arranged in anastomosing cords of cuboidal hepatocytes separated from each other by blood sinusoids lined with endothelial and phagocytic Kupffer cells. The hepatocytes appeared polyhedral in shape with large rounded vesicular nuclei containing prominent nucleoli. The cytoplasm of the hepatocytes appeared eosinophilia (Figures 1A-1C).

Sections of acetaminophen treated rats' revealed markedly disturbed parenchymal architecture of the hepatocytes in the form of degeneration, cytoplasmic vacuolation and hydropic necrosis of hepatocytes. Some hepatocytes displayed acidophilic homogenization of their cytoplasm, while others showed vacuolated cytoplasm and indistinct cell boundaries. The nuclei of these affected cells appeared contracted, pyknotic with condensed chromatin. The central and the portal veins were enlarged and congested exhibited remarkable dilatation and invading infiltrative inflammatory cells were noticed (Figures 1D-1H).

Liver sections of acetaminophen rats treated with N-acetylcysteine revealed marked regeneration and improvement in hepatic cells around the central vein and prominent improvement around the portal area (Figures 1I and 1J). Liver sections of acetaminophen rats treated with curcumin showed more or less architecture similar to the control group where the cellular arrangement around the central vein was brought back. Also, it helped to bring the blood vessels to normal condition observed with the control group (Figures 1K and1L).

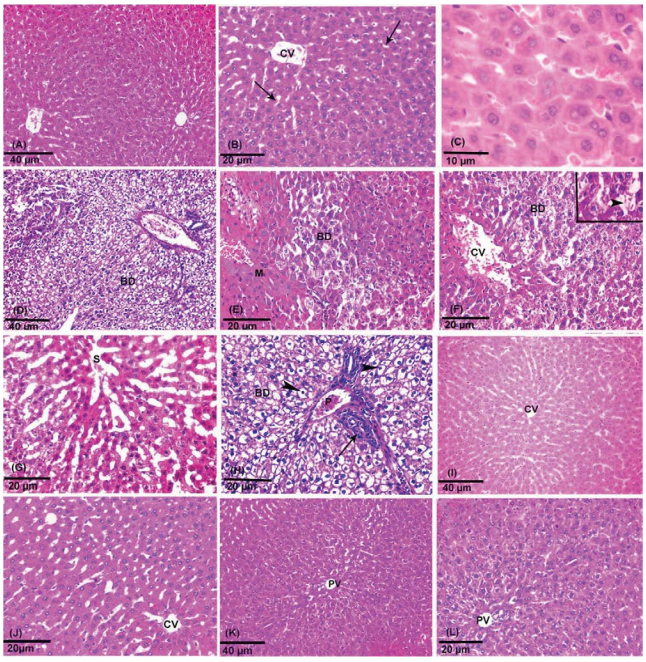


Figure 1: (A-C) Photomicrographs of control rat liver from subgroup (i) (A) Showing normal hepatic architecture (H&E, Mic. Mag. 200X). (**B**) Showing polyhedral hepatocytes with eosinophilic cytoplasm arranged in strands around the central vein (CV), presinusoidal spaces (→) appears between the cords of hepatocytes (H&E, Mic. Mag. 400X). (**C**) Showing polyhedral hepatocytes with rounded vesicular nuclei and prominent nucleoli (→). Notice Kupffer cell (>) (H&E, Mic. Mag. 1000X). (**D-H)** Photomicrographs of liver sections of acetaminophen -treated group exhibit severe damage in the hepatic architecture. Most of hepatocytes fused together forming eosinophilic syncytial (M) masses. Dilatation of central vein (CV), blood sinusoid (S) and portal venule (P) are seen ballooning degeneration of hepatocytes (BD) is seen with vacuolated cytoplasm and indistinct cell boundaries. Notice mononuclear cellular infiltration (→) and apoptotic hepatocytes (>) around the portal venule (H&E, Mic. Mag. D, 200× and E-H, 400X).The inset showing vacuolated hepatocytes with lost nucleus and discontinuous cell boundaries (H&E, Mic. Mag. 1000×). **(I-J**) Photomicrographs of a section of the liver of acetaminophen and N-acetylcysteine treated group showing normal architecture of hepatic lobule and apparently normal looking hepatocytes with central vesicular nuclei around the central vein (CV). (**K-L**) Photomicrographs of liver section in the acetaminophen and curcumin treated group showing normal architecture of hepatocytes, portal venule (PV), and sinusoids (H&E, Mic. Mag. I&K, 200× and J&L, 400X).

The results of histopathologic grading were shown in Table 1. Curcumin had reduced hepatocytes necrosis similar to N-acetylcysteine. As grades 3 and higher was regarded as severe hepatotoxicity, no severe hepatotoxicity was seen in groups 3 and 4; while it was seen in 70% of animals in group 2 (acetaminophen treated group)

Immunohistochemical results

Immunostaining of p53 antigen: In the control group (group I), most of the hepatocytes revealed negative immunostaining reaction for p53. However few scattered cells exhibited faint light brown granules in their cytoplasm (Figure 2A). Acetaminophen treated rats (group II), revealed dark brown granules in their cytoplasm throughout most cells of the liver parenchyma specifically those of the periportal and mid zonal areas (Figure 2B). Acetaminophen rats treated with N-acetylcysteine (group III) and curcumin (group IV) showed reduction in the p53 activity in the hepatocytes to be more or less similar to the control group (Figure 2C and 2D). Quantitative analysis showed that the mean number of p53 positive cells per field in acetaminophen rats significantly increased compared to that of the control group. On the other hand group III& IV showed significant decreases in the number of p53 positive hepatocytes when compared with acetaminophen treated group (Table 2).

Immunoexpression of iNOS: In the control group, the hepatocytes showed weak or no-iNOS immunoreactivity in the cytoplasm of the hepatocytes (Figure 2E). Immunostaining of iNOS was appeared more intense in the cytoplasm of hepatocytes of acetaminophen treated rats compared with the control group (Figure 2F). The immunohistochemical-stained sections of both group III& IV showed that most of the hepatocytes revealed reduced iNOS expression compared with those of the acetaminophen-treated group, with no significant changes when compared with the control group (Figures 2G and 2H). Morphometric analysis confirmed an increase in the mean optical density and area percentage of iNOS immunoreactivity in the acetaminophen treated rat's in-comparison with the control group. However, group III& IV showed non-significant differences in the mean of optical density and area percentage of iNOS immunoreactivity when compared with control group and significant reduction in the mean of optical density and area percentage of iNOS immunoreactivity when compared with the acetaminophen treated group (Table 2).

Biochemical results

Serum enzymes: Table 3 and Figure 3 showed that serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats treated with acetaminophen were significantly elevated by (186.29%, 90.4% and 81.47 %) respectively, as compared to the corresponding control group. However, N-acetylcysteine (group III) significantly inhibited the rise in AST, ALT and ALP by (55.4%, 38.29% and 42.54%) respectively as compared to the corresponding acetaminophen group values. Similarly, curcumin (group IV) significantly inhibited the rise in AST, ALT and ALP by (52.8%, 37.08% and 41.8%) as compared to the corresponding acetaminophen group values.

Histopathologic grading		P. value
Group II	4.2 ± 0.788	
Group III	1.45 ± 0.368	0.023
Group IV	1.15 ± 0.241	0.005

P. value = probability of chance, *P*<0.05 is significant. Group III versus group II and group IV versus group II.

Table 1: Histopathologic grading of liver necrosis in studied groups (mean ± standard deviation).

Status of tissue lipid peroxidation and antioxidant markers: The measured data of lipid peroxidation (LPO), superoxide dismutase (SOD) and glutathione (GSH) in the liver are summarized in Table 3 and Figure 4. Results indicated that LPO concentration end product MAD was significantly increased in the liver ($P < 0.01$) in rats treated with acetaminophen. Pretreatment with N-acetylcysteine (group III) and curcumin (group IV) was very effective in the

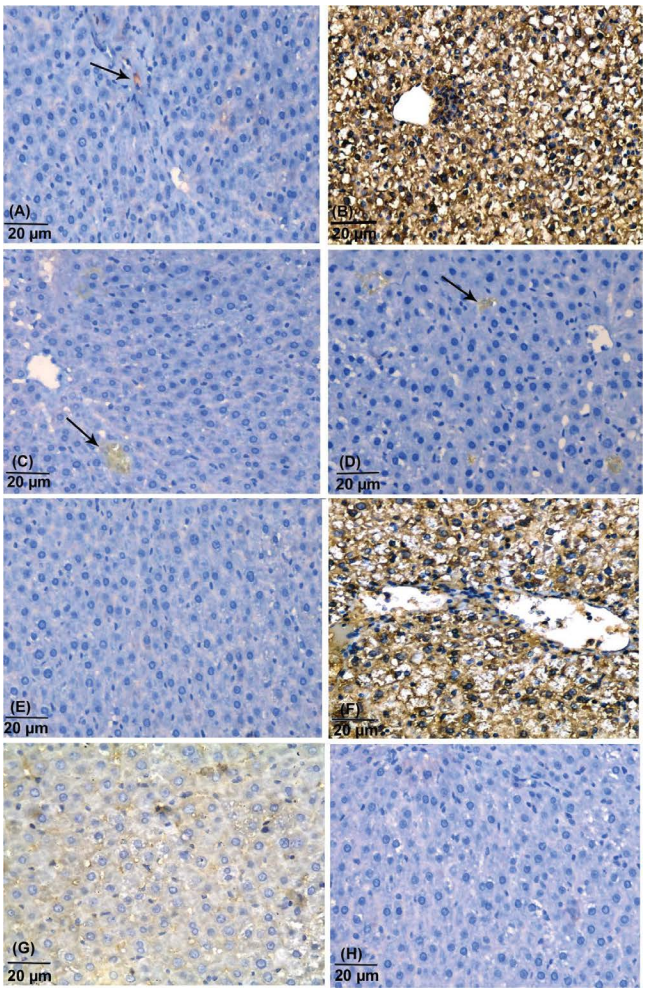


Figure 2: (**A-D**) p53 immunostaining. **(A)** A section of the liver of control group from subgroup (i) showing faint and few cytoplasmic reaction for p53 (→). (**B**) A photomicrograph of a section of the liver of acetaminophen treated group showing strong p53 immunoreactivity in almost all hepatocytes seen. (**C**) A section of the liver of acetaminophen and N-acetylcysteine treated group showing weak p53immunoreativity in some hepatocytes (→). (**D**) A section of the liver of acetaminophen and curcumin treated group showing weak p53 immunoreactivity in some hepatocytes (→) (p53 immunostaining & hematoxylin counterstain, Mic. Mag. 400X). (E-H) iNOS immunostaining. (**E**) A section of the liver of control group from subgroup (i) showing barely detected iNOS proteins in cytoplasm of hepatocytes. **(F)** A photomicrograph of a section of the liver of acetaminophen treated group showing strong expression of iNOS proteins in almost all hepatocytes. **(G)** A section of the liver of acetaminophen and N-acetylcysteine treated group showing weak expression of iNOS in hepatocytes. **(H)** A section of the liver of acetaminophen and curcumin treated group showing barely detected iNOS proteins in cytoplasm of hepatocytes (iNOS immunostaining & hematoxylin counterstain, Mic. Mag. 400X).

Parameters	Control subgroups					Group II	Group III	Group IV
	i	ii	iii	iv	V			
P53 (mean number of cells/field)	1.7 ± 0.48^{NS}	1.9 ± 0.316 ^{NS}	1.8 ± 0.426 ^{NS}	1.9 ± 0.567 ^{NS}	2 ± 0.471 ^{NS}	53.8 ± 10.77 ^{a}P	2.9 ± 0.57 ^{b}P	3.30 ± 0.48 ^{b}P
iNOS (optical density)	0.0738 ± 0.0181 ^{NS}	0.0795 ± 0.0107 ^{NS}	.0771 ± 0.0151 ^{NS}	0.0836 ± 0.0136 ^{NS}	0.0693 ± 0.0122 ^{NS}	6.451 ± 1.098 ^{a}P	0.104 ± 0.028 ^{b}P	0.107 ± 0.019 ^{b}P
iNOS (Area %)	1.425± 0.318 ^{NS}	1.287± 0.313 ^{NS}	1.286 ± 0.225^{NS}	1.2990 ± 0.272 ^{NS}	1.2230 ± 0.220 ^{NS}	89.608 ± 6.11 ^{a}P	1.555 ± 0.242 ^{b}P	1.636 ± 0.234 ^{b}P

Data is expressed as mean ± standard deviation,
NS= non-significant difference between control subgroups tested by one-way ANOVA followed by Post Hoc Tukey's test.
P. value = probability of chance, *P*< 0.05 is significant tested by Student "t" test.
^{a}P = 0.001 *vs* control subgroup i;
^{b}P<0.01*vs* group II.

Table 2: The mean number of p53 positive cells, the mean of iNOS optical density and the mean area percentage of iNOS in the different groups studied.

Parameters	Control subgroups					Group II	Group III	
	i	ii	iii	iv	v			
AST level (IU/L)	34.30 ± 3.86 ^{NS}	36.4 ± 4.57 ^{NS}	37.1 ± 3.87 ^{NS}	38.6 ± 4.19 ^{NS}	38.5 ± 3.50 ^{NS}	98.2 ± 8.23 ^{a}P	43.8 ± 7.89 ^{b}P	45.4 ± 6.51 ^{b}P
ALT level (IU/L)	165.5 ± 10.69 ^{NS}	171.9 ± 11.81 ^{NS}	169.9 ± 12.85 ^{NS}	173.4 ± 12.56 ^{NS}	177.4 ± 13.59 ^{NS}	315.2 ± 12.86 ^{a}P	194.5 ± 11.94 ^{b}P	198.3 ± 14.12 ^{b}P
ALP level (IU/L)	178.6 ± 12.01 ^{NS}	190.5 ± 12.20 ^{NS}	185.7 ± 11.47 ^{NS}	183.1 ± 14.87 ^{NS}	190.5 ± 11.46 ^{NS}	324.1 ± 21.07 ^{a}P	186.2 ± 10.16 ^{b}P	188.6 ± 13.1 ^{b}P
MDA level(nmol/g tissue protein)	53.8 ± 2.78 ^{NS}	51.8 ± 3.19 ^{NS}	52.6 ± 3.77 ^{NS}	51.9 ± 3.03 ^{NS}	51.7± 2.98 ^{NS}	103.2 ± 5.90 ^{a}P	60.9 ± 5.25 ^{b}P	58.7 ± 4.32 ^{b}P
GSH level (µmol/g tissue protein)	6.54 ± 1.13 ^{NS}	7.09 ± 0.65 ^{NS}	6.73 ± 0.72 ^{NS}	7.11± 0.80 ^{NS}	6.89 ± 0.72 ^{NS}	2.35 ± 0.77 ^{a}P	7.99 ± 0.98 ^{b}P	8.11 ± 0.75 ^{b}P
SOD level (units/mg tissue)	5.16 ± 0.47 ^{NS}	5.09 ± 0.46 ^{NS}	5.19 ± 0.56 ^{NS}	5.26 ± 0.65 ^{NS}	5.31 ± 0.47 ^{NS}	1.79 ± 0.49 ^{a}P	6.04 ± 0.49 ^{b}P	5.89 ± 0.64 ^{b}P

Data is expressed as mean ± standard deviation,
NS= non-significant difference between control subgroups tested by one-way ANOVA followed by Post Hoc Tukey's test.
P. value = probability of chance, *P*< 0.05 is significant tested by Student "t" test.
^{a}P = 0.01*vs* control group i;
^{b}P<0.01*vs* group II.

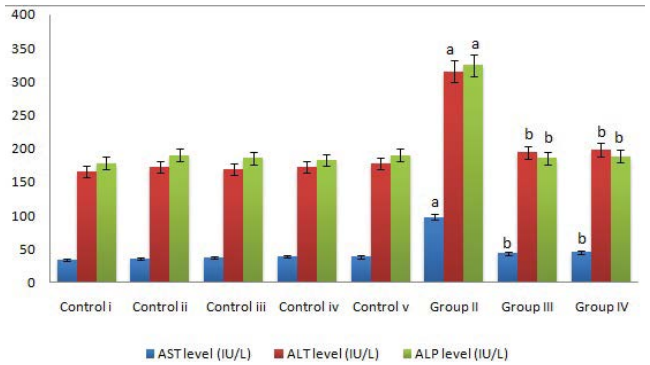
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; MDA: Malondialdehyde; GSH; Glutathione; SOD: Superoxide dismutase.

Table 3: Serum levels of liver injury markers and measurement of some oxidative stress factors in the different groups studied.

prevention of oxidative damage induced by acetaminophen, which resulted in significantly lower MAD concentration when compared with acetaminophen -treated group. Acetaminophen treatment decreased the levels SOD and GSH significantly (P<0.05) in the liver. By contrast, increased levels of GSH and SOD were observed in N-acetylcysteine plus acetaminophen treated group and in curcumin plus acetaminophen -treated group when compared with acetaminophen treated group.

Discussion

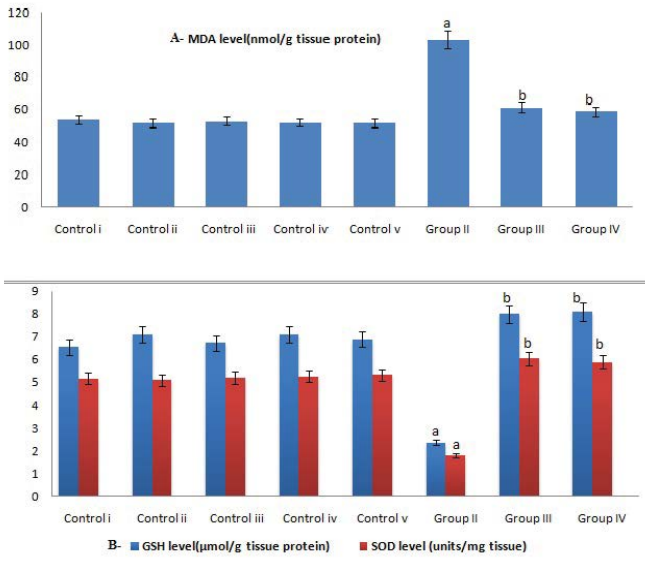
In the present study the ability of curcumin to protect the liver against the oxidative stress and the hepatocellular injury that follows an overdose of acetaminophen were studied and compared with N-acetylcysteine. In this study, acetaminophen caused degeneration, hydropic necrosis of hepatocytes with enlargement and congestion of central and the portal veins. Marked elevation in the serum levels of liver injury markers and disturbances in the oxidative stress markers were recorded. However, administration of acetaminophen with curcumin or with N-acetylcysteine markedly reduced both hepatic histo-architecture and biochemical abnormalities.



Non-significant difference between control subgroups tested by one-way ANOVA followed by Post Hoc Turkey's test.
^{a}P = 0.01*vs* control group i;
^{b}P< 0.01*vs* group II. Tested by Student "t" test.

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

Figure 3: Serum levels of liver injury markers in the different groups studied.



Non-significant difference between control subgroups tested by one-way ANOVA followed by Post Hoc Tukey's test.
^{a}P = 0.01*vs* control group i;
^{b}P< 0.01*vs* group II, tested by Student "t" test.
MDA: Malondialdehyde; GSH; Glutathione; SOD: Superoxide dismutase.

Figure 4: Measurement of some oxidative stress factors in the different groups studied.

Drug induced liver injuries are widespread and has become the disposing cause of acute liver failure and transplantation in many countries and mimic all forms of acute and chronic liver disease. It is thought that the liver is the target organ for acetaminophen toxicity because this is primarily where the drug is detoxified [30].

Under normal conditions, acetaminophen is mainly metabolized by going through sulfation and glucuronidation. It has been proposed that a small amount of the drug goes through the cytochrome P450 mixed function oxidase system and is metabolized into the reactive intermediate N-acetyl-P-benzo quinoneimine (NAPQI) which is in turn detoxified by reaction with glutathione [31]. When large doses of acetaminophen are consumed, the detoxification pathways become saturated and result in depletion of hepatocellular GSH. Once GSH is consumed, any persisting NAPQI will covalently attach to cellular proteins and provoke oxidative stress, lipid peroxidation, DNA fragmentation and mitochondrial dysfunction finally leads to massive hepatocyte necrosis, liver damage or death [32,33]. In agreement with the results of other studies [33,34] acetaminophen overdose treatment in this study showed significantly increase in serum transaminase, hepatic malondialdehyde (MDA), and decrease in hepatic GSH and SOD. Histological examination showed a hepatocellular necrosis as manifested by swelling of the hepatocytes with cytoplasmic vacuolations and dark nuclei.

Liver enzymes ALT, AST, and ALP are often used as markers of hepatic injury as their increase indicates liver damage [35]. The marked release of transaminases into the circulation is indicative of severe damage to hepatic tissue membranes during paracetamol intoxication [36]. This was consistent with the results of previous investigators who studied the effect of chronic paracetamol ingestion on liver damage [37]. The present biochemical study showed that N-acetylcysteine significantly inhibited the rise in AST, ALT and ALP by (55.4%, 38.29% and 42.54%) respectively as compared to the corresponding acetaminophen group values. Similarly, curcumin significantly inhibited the rise in AST, ALT and ALP by (52.8%, 37.08% and 41.8%) as compared to the relative acetaminophen group values. The previous results are in accordance with the results of previous researchers who demonstrated that these increases in liver enzymes were reduced by the administration of curcumin [38] and N-acetylcysteine [39].

To assess lipid peroxidation MDA was used as a marker and reduced GSH and SOD as indicators of hepatoprotectivity for cells [40]. In this study the oxidative damage caused by acetaminophen overdose was significantly attenuated by administrating curcumin and N-acetylcysteine. The most accepted explanation for the protective actions of N-acetylcysteine is that it serves as a source of L-cysteine for GSH synthesis and, hence, it can help the detoxification of NAPQI before this reactive metabolite can initiate hepatic injury [41,42] and acts as a scavenger of ROS that it supports mitochondrial energy metabolism [43]. Likewise to N-acetylcysteine, curcumin could save against free radical mediated oxidative stress by scavenging for free radicals that restricts lipid peroxidation incorporated in membrane damage. In this study the GSH consumption caused by acetaminophen overdose was significantly attenuated by administrating curcumin. The protective action of curcumin can be explained by GSH inducer through induction of glutathione reductase enzyme system [38,44].

In the present study liver sections of acetaminophen rats treated with N-acetylcysteine revealed marked regeneration and improvement in hepatic cells around the central vein and prominent improvement around the portal area this indicated that N-acetylcysteine is regarded as the antidote of choice for treating acetaminophen overdoses. Also, in this study curcumin had a similar effect on liver morphology; these results are in agreement with Somanawat et al. [38]. They concluded that curcumin prevents acetaminophen induced hepatitis by decreasing oxidative stress, liver inflammation and restoration of glutathione. These observations were well correlated with the biochemical findings and gives clear evidence that there was not only an improvement in the liver functions with the treatment of curcumin and N-acetylcysteine, but also in the hepatic architecture.

Accumulating evidence suggests that hepatocyte apoptosis plays a critical role in acetaminophen -induced hepatic injury, although the mode of cell death imposed by acetaminophen is still controversial [5]. Acetaminophen induced apoptosis is observed in primary hepatocytes [45] and also in livers of mice treated with toxic doses of acetaminophen [46]. Cells committed to die through p53-dependent apoptosis typically trace the mitochondrial pathway, even though p53 can also inflect cell death through death receptors. Besides, most evidence indicates that the key contribution of p53 in apoptosis is principally dependent on transcriptional activity. p53 has the capacity to trigger transcription of various proapoptotic genes, involving those encoding members of the Bcl-2 family [47,48] thus promoting caspase activation. Based on these concepts, we found that apoptotic hepatocytes were significantly increased in the liver of rats after acetaminophen treatment and the hepatocyte apoptosis was significantly reduced by both curcumin and N-acetylcysteine treatment as detected by quantitative analysis of the immunohistochemical study of p53 antigen. A recent study showed that curcumin exerts a potent anti-apoptotic effect by decreasing the expression of pro-apoptotic genes (Bax, caspase-3) and increasing the expression of anti-apoptotic genes (Bcl-XL) [42]. NAC attenuates lipopolysaccharide induced apoptotic liver injury via its strong ROS scavenging and anti-apoptotic effects [46].

Immunoexpression of iNOS in this study revealed reduced iNOS expression in groups treated with N-acetylcysteine and curcumin compared with those of the acetaminophen-treated group, with no significant changes when compared with the control group, these results are in harmony with Gardner et al. [49]. They reported that toxic doses of acetaminophen to rats induced iNOS in the centrilobular hepatocytes and treatment of rats with the iNOS inhibitor decreased hepatotoxicity. Another study showed that acetaminophen toxicity was decreased in iNOS knockout mice compared to the wild-type mice [50]. It has been documented in other studies also, that curcumin inhibits iNOS gene expression in the livers of lipopolysaccharide injected mice [51], thus suppress the release of nitric oxide by down-regulating iNOS and enhance cellular antioxidant defenses.

From the above result, we confirm that the N-acetylcysteine is the ideal treatment in the acetaminophen overdose toxicity, but the other antioxidant like curcumin has the same effect and has the same role in the treatment of acetaminophen overdose toxicity and its role at least as important as N-acetylcysteine. Curcumin could attenuate acetaminophen-induced liver injury by its antiapoptotic effect, decrease oxidative stress, reduce liver inflammation, restore hepatic GSH, and normalize the hepatic histo-architecture.

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