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RESEARCH ARTICLE

A COMPARATIVE STUDY OF HEPATOPROTECTIVE EFFECT OF CURCUMIN WITH N-ACETYL CYSTEINE AND THEIR SIMULTANEOUS ADMINISTRATION IN ACETAMINOPHEN INDUCED HEPATOTOXICITY

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ABSTRACT

Introduction: The available therapeutic option in acetaminophen induced hepatotoxicity, N-acetyl cysteine (NAC) has potential side effects. Curcumin has shown hepatoprotective effect in animal models.

Objective: To compare hepatoprotective efficacy of curcumin with NAC and their simultaneous administration in acetaminophen induced hepatotoxicity.

Materials and Methods: Hepatotoxicity was induced in Wistar rats by acetaminophen (2000 mg/kg). NAC (300 mg/kg) was used as standard control in study. Curcumin was administered in doses of 300, 600 and 900 mg/kg. After evaluation of best maximal effective dose of curcumin, drugs were co-administered in half doses and full doses of each in different groups. Hepatic transaminases, liver tissue MDA and GSH content, histological examination were variables assessed.

Results: Lowering of enzyme levels in curcumin and co-administration of curcumin and NAC group were similar to NAC treated group. Co-administration of curcumin and NAC group had comparable levels of MDA to curcumin treated alone groups. Co-administration of NAC and curcumin had significantly higher GSH compared to NAC used alone. Histopathological examination scores in these groups were comparable.

Conclusion: Co-administration of NAC and curcumin in half doses of each drug, in acetaminophen induced hepatotoxicity in Wistar rats, has a comparable hepatoprotective effect to NAC or curcumin used alone.

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INTRODUCTION

Ever since 1970, acetaminophen overdose has been alarmingly increased in many countries and has been an important cause for hospital admission in developed countries like in United Kingdom, United States, Europe and Australia. (Sheen *et al*., 2002) The most important outcome of acetaminophen overdose is acute liver injury, which may be fatal. Current standard of treatment is N acetyl cysteine (NAC), as it supplies sulfhydryl groups for regeneration of reduced glutathione. (Sheen *et al*., 2002) Liver transplantation as an option is very limited, owing to limited skillful resources and high costs. Data of the prevalence of acetaminophen overdose is available for the developed nations. However, in the developing nations and Asian populations the data has been scarce. In a study by Marzilawati *et al* in Malaysia, the incidence of acetaminophen poisoning in ethnic Indians has been reported to be 3.8%. (Marzilawati *et al*., 2012) Acetaminophen poisoning is of concern in the developing nations, as the fatality rate owing to drug over dosage has been estimated to be about 15 times higher than the developed nations. (Anthony and Kulkarni, 2012) In India, acetaminophen is easily available as an OTC drug and is readily purchased without a prescription. It is also frequently prescribed by the physicians. Indian studies regarding prevalence of acetaminophen poisoning are lacking. In a study by Anthony *et al* in Bengaluru, the hospital admission owing to this poisoning has been reported to be about 0.03% of the total admissions owing to drug over dosage. (Anthony and Kulkarni, 2012) The acetaminophen overdose causes significant mortality and morbidity in patients, with huge financial costs of treatment and hospital admissions. (Sheen *et al*., 2002) There is a need for cheaper drugs with better efficacy than NAC. No drug has been approved for use

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in acetaminophen induced liver failure other than NAC. NAC has numerous adverse effects, mainly anaphylactoid reactions which may be fatal, others include cerebral edema and hyponatremia. (Nambiar, 2012) Out of the number of drugs studied, curcumin has shown strong potential as treatment against acetaminophen induced liver injury in numerous studies, owing to its anti-oxidant activity and anti-inflammatory activity. (Somanawat *et al*., 2013; Li *et al*., 2013) However, studies directly comparing curcumin with NAC in acetaminophen induced hepatotoxicity are lacking. Curcumin may have better protective effect as it is a dietary supplement and not associated with considerable adverse effect. (Lao *et al*., 2006) Hence we decided to evaluate the effect of curcumin in acetaminophen induced hepatotoxicity and compare the protective effect with the current standard i.e. NAC. Additionally, on extensive literature search we found that studies with use of combination of curcumin with NAC were lacking. (Kheradpezhouh *et al*., 2010) Only one study was conducted, that too used only one lower dose combination. Hence, we decided to evaluate the multiple doses of curcumin in addition we also decided to evaluate dose ranging combination of curcumin with NAC in order to appreciate additional benefits, if any.

Objective

Compare the hepatoprotective efficacy of curcumin with N-acetyl cysteine and their simultaneous administration in a standardised model of acetaminophen induced hepatotoxicity in rats.

MATERIALS AND METHODS

Experimental animals

Following permission from Institutional Animal Ethics Committee, 60 Wistar rats of either sex, each weighing between 180-220 gm, randomly bred in Central Animal House of institution were incorporated in study. Animals were divided into 10 groups of 6 rats each (Table 1)

Husbandry conditions

Animals were housed in the Central Animal House of Institute, in an air conditioned area with 12 – 15 filtered fresh air changes, temperature 22 ± 3° C, relative humidity 30 – 70%. Four rats per cage were housed in cages during study. Cages had a stainless steel top grill having facilities for food and drinking water in polypropylene bottles with stainless steel sipper tube. Standard rat feed was provided ad libitum. Aqua-guard pure drinking water was provided to animals.

Study drugs and administration

Acetaminophen and NAC were dissolved in distilled water and given orally, while curcumin was dissolved in corn oil for oral administration to rats.

Study design and procedure

The initial evaluation for all rats was same. Rats were kept fasting for 12 hours prior to administration of drug. Just before feeding the drug, baseline weight of rat was measured and blood was withdrawn from retro-orbital vein for measuring of baseline liver function tests. After administering acetaminophen in toxic doses to the rats, standard or test drug or combination were administered to the rat after 2 hours in respective group. Treatment was provided to the rats 8 hourly. After 48 hours (6 doses of treatment) of drug administration, blood was collected again from rats from retro-orbital vein for measuring liver function tests. Thereafter, rats were sacrificed by cervical dislocation. Abdomen of rat was dissected, liver cut out and immediately immersed in cold saline buffered solution. The liver was cut into 3 parts:

1. Part was immersed in 10 % formalin solution for histopathological staining and analysis.
2. 1 gm was used for measuring MDA levels in liver tissue.
3. 1 gm was used for measuring GSH levels in liver tissue.

The rats in normal control group were used for assessing the normal histopathology and liver function parameters.

Following parameters were assessed for toxicity

The variables assessed were as follows:

a) Liver function parameters (These parameters were measured before and 48 hours after administering acetaminophen to rats)

1.Serum Bilirubin (total)

2.Alanine transaminase (SGPT)

3.Aspartate transaminase (SGOT)

b) Indicators of Oxidative damage

1. Hepatic Glutathione (GSH) using Owens method. (Owens and Belcher, 1965)
2. Hepatic Malonaldialdehyde (MDA) using Okhawa *et al* method. (Okhawa *et al*., 1979)

c) Histopathological examination of liver after sacrificing the animals. Scoring was performed using Brunt *et al* scoring. (Brunt *et al*., 1999)

Statistical analysis

Statistical analysis of data was done using the SPSS version 21 software for Windows. The normality of the data was assessed using the Kolmogorov-Smirnov test. The parametric data between two groups was compared using unpaired t test while the non-parametric data between two groups was compared using Mann-Whitney test. Between the groups, the data which was normally distributed was compared using one-ANOVA test followed by post hoc Tukey's test while the data which was not normally distributed was compared using Kruskal-Wallis test. The histopathological scores between the groups were compared using Kruskal-Wallis test. The level of significance for each comparison in the analysis was calculated at 0.05.

RESULTS

Part I: Assessment of variables in the disease control group compared to normal control and vehicle control groups (post induction) (Table 2)

The total and direct serum bilirubin between all the groups were not different.

Table 1.

Groups	Inducing agent administered	Treatment given
1.(Normal control)	Distilled water	Nil
2.(Vehicle control)	Corn Oil	Nil
3.(Disease control)		Nil
4.(Standard control)		N-Acetyl cysteine (300 mg/kg)(Megnathan *et al*., 2011)
5.(C300)		Curcumin (300 mg/kg)(Somanawat *et al*., 2013)
6.(C600)		Curcumin (600 mg/kg)(Somanawat *et al*., 2013)
7.(C900)	Acetaminophen	Curcumin (900 mg/kg)
8.	(2000 mg/kg) (Megnathan	Curcumin* + N-Acetyl cysteine (150 mg/kg)
9.	*et al*., 2011)	Curcumin^ + N-Acetyl cysteine (300 mg/kg)
10.		Curcumin* + N-Acetyl cysteine (200 mg/kg) intraperitoneally (Kheradpezhouh *et al*., 2010)

*Half dose of curcumin which is maximally effective in Groups 5, 6, 7.
^Full dose of curcumin which is maximally effective in Groups 5, 6, 7.

Table 2. Part I - Results of Standardisation

Variable	Normal control	Vehicle control	Disease control
Total serum bilirubin (mg/dl)[a]	0.25 (0.1-0.6)	0.2 (0.2-0.3)	0.25 (0.1-0.4)
Direct serum bilirubin (mg/dl)[a]	0.15 (0.05-0.3)	0.1 (0.06-0.2)	0.1 (0.1-0.2)
SGOT (mg/dl)[b]	82.21±4.73	78.02±9.17	302.47±22.24$^{****\#\#\#\#}$
SGPT (mg/dl)[b]	39.39±7.85	35.85±3.33	128.07±23.49$^{****\#\#\#\#}$
Liver MDA (µmol/gm)[b]	162.67±12.03	162.67±25	263.75±5.22$^{****\#\#\#\#}$
Liver GSH (µmol/gm)[b]	1.36±0.2	1.63±0.26	0.55±0.08$^{****\#\#\#\#}$
Histopathological score[c]	0	0	1 (1-2)$^{**\#\#}$
Liver weight per 100 gm of body weight (g/100g)[b]	3.61±0.32	3.57±0.37	4.68±0.59$^{**\#\#}$
Liver volume per 100 gm of body weight (ml/100g)[b]	3.47±0.34	3.58±0.52	4.84±0.83$^{**\#\#}$

[a]Wilcoxon signed rank test, [b]One way Anova (post hoc Tukey's), [c]Kruskal-Wallis test
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 Normal control vs Disease control
#p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 Vehicle control vs Disease control

Part II: Assessment of variables in the standard control group compared to the disease control group

Table 3.

Variable	Disease control	Standard Control
SGOT (mg/dl)[a]	302.47±22.24	220.48±28.22****
SGPT (mg/dl)[a]	128.07±23.49	82.72±20.44**
Liver MDA (µmol/gm)[a]	263.75±5.22	235.36±24.1*
Liver GSH (µmol/gm)[a]	0.55±0.08	1.15±0.12****
Histopathological score[b]	1 (1-2)	1 (0-1)
Liver weight per 100 gm of body weight (g/100g)[a]	4.68±0.59	4.97±0.5
Liver volume per 100 gm of body weight (ml/100g)[a]	4.84±0.83	4.1±0.42

[a]Unpaired t test, [b]Mann-Whitney test
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 Normal control vs Disease control

Part III: Evaluation of best effective dose of curcumin in the hepatotoxicity induced groups in comparison to the disease control group

Table 4.

Variable	Disease control	C300	C600	C900
SGOT (mg/dl)[a]	302.47±22.24	287.74±34.5	209.25±26.64$^{**\#}$	242.04±75.61
SGPT (mg/dl)[a]	128.07±23.49	122.37±34.39	110.51±12.48	112.78±43.47
Liver MDA (µmol/gm)[a]	263.75±5.22	235.38±13.22****	205.54±6.66$^{****\#\#\#\#}$	198.1±5.09$^{****\#\#\#\#}$
Liver GSH (µmol/gm)[a]	0.55±0.08	0.57±0.05	1.28±0.05$^{****\#\#\#\#}$	1.24±0.14$^{****\#\#\#\#}$
Histopathological score[b]	1 (1-2)	1 (0-2)	0 (0-1)**	0 (0-1)**
Liver weight per 100 gm of body weight (g/100g)[a]	4.68±0.59	4.1±0.45	3.68±0.34**	3.53±0.31**
Liver volume per 100 gm of body weight (ml/100g)[a]	4.84±0.83	3.8±0.94	2.8±0.54****	2.72±0.27****

[a]One way Anova (post hoc Tukey's), [b]Kruskal-Wallis test
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs Disease control
#p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 vs C300

Part IV: Comparison of variables in the curcumin and co-administration groups with the standard treatment group

Concluding from the results of part 3, the co-administration groups were given the following doses of curcumin and NAC

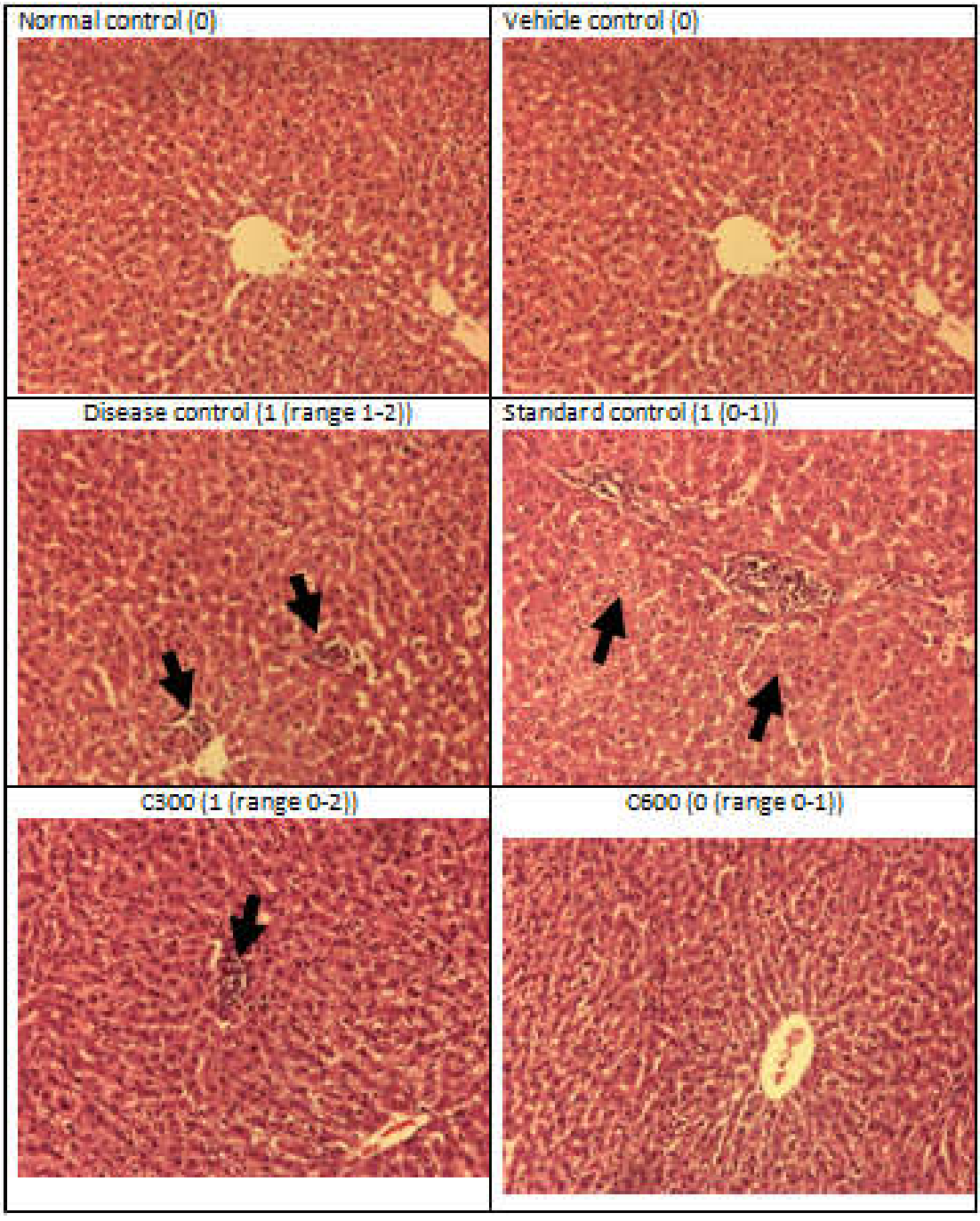
Group	Dose of curcumin	Dose of NAC	Group code
Group 8	300 mg/kg	150 mg/kg	NAC150+C300
Group 9	600 mg/kg	300 mg/kg	NAC300+C600
Group 10	300 mg/kg	200 mg/kg (intraperitoneally)	NAC200+C300

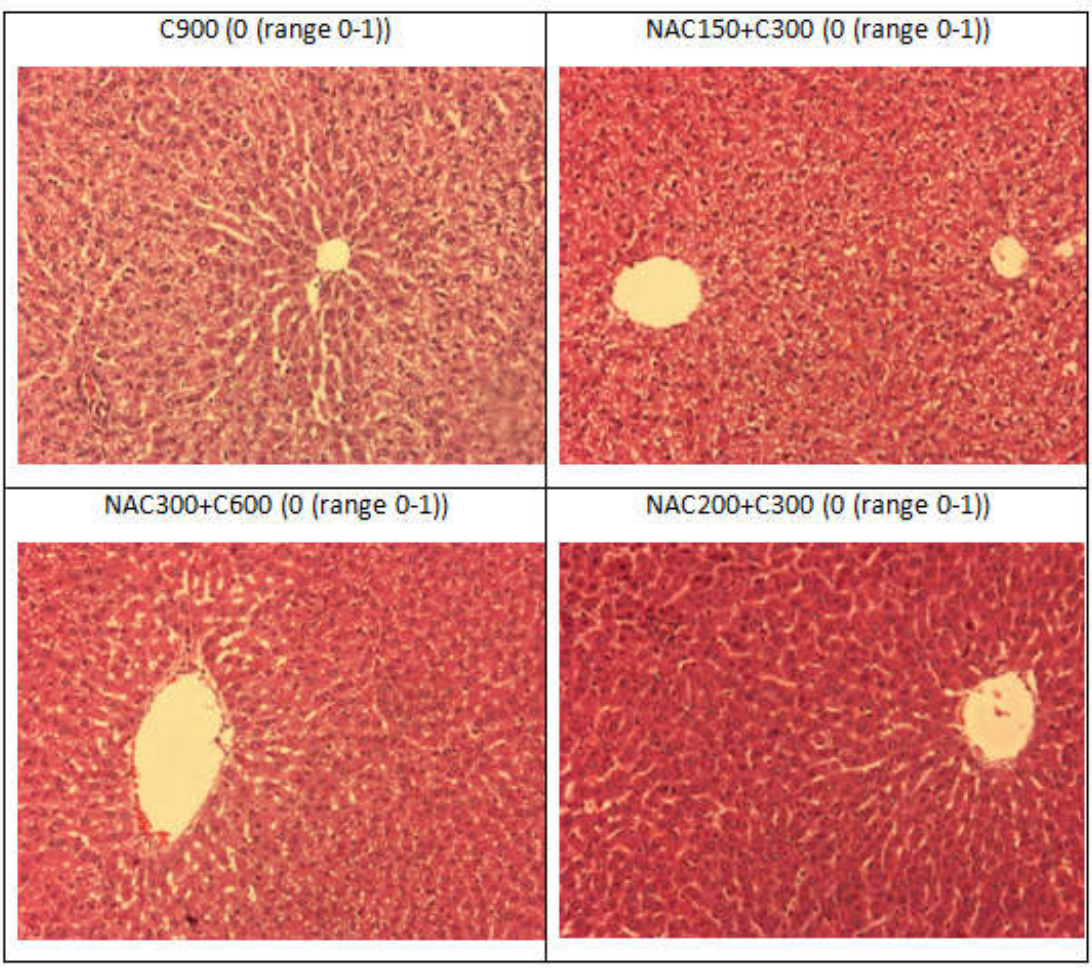
Table 5.

Variable	Standard control	C600	NAC150+C300	NAC300+C600	NAC200+C300
SGOT (mg/dl)[a]	220.48±28.22	209.25±26.64	192.17±27.36	203.35±12.31	233.02±68.77
SGPT (mg/dl)[a]	82.72±20.44	110.51±12.48	92.58±4.68	87.58±7.55	112.42±38.13
Liver MDA (µmol/gm)[a]	235.36±24.1	205.54±6.66**	274.11±7.05$^{**\#\#\#\#}$	248.29±14.74$^{\#\#\#\#}$	212.68±7.58
Liver GSH (µmol/gm)[a]	1.16±0.12	1.28±0.05	1.38±0.1**	1.29±0.05	1.31±0.06*
Histopathological score[b]	1 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
Liver weight per 100 gm of body weight (g/100g)[a]	4.97±0.5	3.68±0.34****	4.01±0.31**	3.6±0.36****	3.35±0.3**
Liver volume per 100 gm of body weight (ml/100g)[a]	4.1±0.42	2.8±0.54**	3.59±0.3	3.45±0.67	3.11±0.46*

[a]One way Anova (post hoc Tukey's), [b]Kruskal-Wallis test
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs Standard control
$^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001, $^{\#\#\#\#}$p<0.0001 vs C600

Comparison of Histopathological examination of liver (Brunt et al score) (Median-range) (10x magnification) (Haematoxylin eosin staining) (Arrows: Mild Focal Necrosis)





After comparing the variables in the curcumin only treated groups it was found that the variables in the C600 and C900 group showed consistent significant differences with that of the disease control group, and no significant difference was found in the variables between the C600 and C900 groups. Hence, the best effective dose in acetaminophen induced hepatotoxicity is at 600 mg/kg and was used for further combination doses as mentioned in the materials and methods section (Table 4).

DISCUSSION

In the present study, we found curcumin in the dose of 300 mg/kg co-administered with NAC 150 mg/kg produced hepatoprotective effect which was comparable to NAC 300mg/kg or curcumin 600mg/kg administered alone. Curcumin has been evaluated in various doses in acetaminophen induced hepatotoxicity (50 mg/kg (Yousef *et al*., 2010; Farghaly and Hussein, 2010), 400 mg/day (Soliman *et al*., 2014) p.o.), CCl4 induced hepatotoxicity (200 mg/kg p.o.) (Reyes-Gordillo *et al*., 2007; Morsy *et al*., 2012) and lindane induced oxidative stress in liver (100 and 200 mg/kg p.o.) (Singh and Sharma, 2011) in experimental studies in Wistar rats. Curcumin (50 mg/kg p.o.) administered for 15 days orally in Wistar rats, showed hepatoprotective effect in the study by Yousef *et al*. as observed by alleviation of alteration of liver function tests like SGOT, SGPT, alkaline phosphatase, increased liver tissue TBARS and reduction in the liver GSH content and attenuation of necrotic changes in the liver due to acetaminophen (650 mg/kg) given on 15th day. (Yousef *et al*., 2010) Soliman *et al* showed the hepatoprotective effect of curcumin (400 mg/day for 7 days) when administered with acetaminophen (500 mg/kg on 7th day) prevented the acetaminophen induced increase in SGOT, SGPT, serum urea, serum albumin and liver MDA activity, and also prevented acetaminophen induced decrease in catalase activity and improved liver histology. (Soliman *et al*., 2014) In a study by Farghaly *et al*, curcumin in a daily oral dose of 50 mg/kg in adult albino rats for 10 days before or after administration of 500 mg/kg of acetaminophen for 5 days, showed hepatoprotection by preventing the acetaminophen induced increase in liver TBARS levels and depletion of GSH in the liver and by increasing the activity of antioxidant enzymes in liver. (Farghaly and Hussein, 2010) Kheradpezhouh *et al* observed the hepatoprotective effect of curcumin in intraperitoneal doses of 50 and 100 mg/kg in acetaminophen (750 mg/kg) induced hepatotoxicity in Sprague-Dawley rats at 7 days, as observed by reduced hepatic transaminase levels, increase in glutathione peroxidase levels, lower MDA levels and normal histology of liver which are altered in acetaminophen overdose. (Kheradpezhouh *et al*., 2010) Li *et al* observed the hepatoprotective effect of curcumin in mice in dose of 10 and 20 mg/kg when administered 2 hours before and after administration of acetaminophen toxic dose at 16 hours, as observed by reduction in SGOT levels and tissue MDA levels. (Li *et al*., 2013) In a study by Somanawat *et al*, curcumin (200 and 600 mg/kg p.o.) reduced SGOT, SGPT, IL-

12, IL-18 and liver MDA levels and increased the liver GSH content compared to the untreated groups at 24 hours of administering 400 mg/kg acetaminophen in mice, thus, proving curcumin as a hepatoprotectant. (Somanawat *et al*., 2013) Kalantari *et al* evaluated hepatoprotective effect of curcumin in various doses (200, 400, 800 and 1000 mg/kg p.o.) in mice and found that curcumin reduces the hepatic transaminase levels which are increased with 750 mg/kg of acetaminophen and restores normal histology of liver after 24 hours of drug administration. (Kalantari *et al*., 2007) A study in adult male rabbits by Sayed *et al*. curcumin (50 and 100 mg/kg/day p.o.) administered along with acetaminophen (50 mg/kg/day) daily for 15 days, produced a hepatoprotective effect as observed with reduced SGOT and SGPT levels and normal histology in comparison to acetaminophen only group. (Sayed and El-Kordy, 2014) The maximal hepatoprotective effect of curcumin in the study by Kalantari *et al* was observed at 800 mg/kg. (Kalantari *et al*., 2007) In the current study, the maximal hepatoprotective effect of curcumin was found in a dose of 600 mg/kg in Wistar rats when acetaminophen was given in a dose of 2000 mg/kg.

While NAC in the dose of 150 mg/kg and 300 mg/kg mg/kg in Wistar Rats has been proved to be protective in acetaminophen induced hepatotoxicity in terms of reduction of hepatic transaminase levels, alkaline phosphatase, lactate dehydrogenase and MDA levels, and preservation of normal histology of liver. (Megnathan *et al*., 2011; Yousef *et al*., 2010) Indicators of hepatotoxicity of acetaminophen in Wistar rats that we selected were SGOT and SGPT enzyme levels, liver tissue MDA and GSH content, and histological examination of liver tissue. In normal doses, 4% of acetaminophen is metabolized by cytochrome P450 enzymes, produces a reactive metabolite, NAPQI which is conjugated by glutathione to stable products. (Ben-Shachar *et al*., 2012) However, in toxic doses the other metabolism pathways like glucuronidation and sulfation are saturated and so more amount of acetaminophen is metabolized by P450 enzymes. (Ben-Shachar *et al*., 2012) After some time, the glutathione stores (GSH) in the liver are depleted and more NAPQI is generated. (Ben-Shachar *et al*., 2012)NAPQI leads series of chain reactions forming adducts with different cell constituents like proteins of the cytoplasm as well as mitochondria. (Ben-Shachar *et al*., 2012; Jaeschke *et al*., 2014) The free radicals generated lead to oxidation of the lipid cell membrane, thereby, increasing the products of lipid peroxidation. (Ben-Shachar *et al*., 2012) MDA is a major reactive aldehyde produced as a result of peroxidation of the biological membranes, and indicates tissue damage due to chain reactions of the free radicals. (Siddique *et al*., 2012) Ultimately, mitochondrial dysfunction ensue and cell membrane is damaged leading to necrosis of the liver cell. (Ben-Shachar *et al*., 2012; Jaeschke *et al*., 2014) Raised SGOT and SGPT are indicators of liver cell injury. (Gowda *et al*., 2009) In the present study, we found that in acetaminophen induced hepatotoxicity an increase in SGOT and SGPT activity was observed which was lowered by NAC and curcumin, as well as their co-administration. Our result is in accordance with studies by Yousef *et al*, Soliman *et al*, Li *et al*, Kheradpezhouh *et al*, Somanawat *et al*, Kalantari *et al* and Sayed *et al* with who observed fall in SGOT and SGPT by curcumin in acetaminophen induced hepatotoxicity. (Somanawat *et al*., 2013; Li *et al*., 2013; Kheradpezhouh *et al*., 2010; Yousef *et al*., 2010; Soliman *et al*., 2014; Kalantari *et al*., 2007; Sayed and El-Kordy, 2014) Comparative study of curcumin, NAC and their co-administration was previously done only by Kherapezhouh *et al*. (2010) In the study by Kheradpezhouh *et al*, a single co-administration dose (so as to study synergistic effect of curcumin and NAC) with lowest dose of curcumin (25 mg/kg) and NAC (200 mg/kg) was used. (Kherapezhouh *et al*., 2010) NAC (800 mg/kg) and co-administration (curcumin 25 mg/kg and NAC 200 mg/kg) reduced SGOT and SGPT levels in their study. (Kherapezhouh *et al*., 2010) Effect of co-administration with respect to hepatic enzymes in the Kheradpezhouh *et al* study are similar to that in our study. NAC, curcumin as well as their co-administration prevent necrosis of the liver cells in acetaminophen toxicity, thereby, preventing an increase in the levels of SGOT and SGPT.

Curcumin and NAC reduced the acetaminophen induced increase in liver MDA levels in the present study. Reduction of liver MDA levels in acetaminophen induced hepatotoxicity by curcumin was observed in the studies by Farghaly *et al*, Yousef *et al*, Li *et al*, Kheradpezhouh *et al*, Somanwat *et al*, and Soliman *et al* which was similar to the present study. (Somanawat *et al*., 2013; Li *et al*., 2013; Kheradpezhouh *et al*., 2010; Yousef *et al*., 2010; Soliman *et al*., 2014) In the present study, the co-administration of NAC and curcumin failed to lower the liver MDA levels as effectively as NAC or curcumin used alone. However, when the route of administration of NAC (intraperitoneally) and curcumin (oral) was different, the liver MDA levels were comparable to that of NAC or curcumin used alone. Co-administration of NAC and curcumin in the Kheradpezhouh *et al* produced liver MDA levels similar to that of NAC or curcumin used alone, which is in contrast to that in our study, except in the group with intraperitoneal route administration in our study. (Kheradpezhouh *et al*., 2010) In the study by Kheradpezhouh *et al*, both the drugs were co-administered by the intraperitoneal route. (Kheradpezhouh *et al*., 2010) The differential hepatoprotective effect of co-administration depending on the different routes of administration of drugs cannot be explained and needs to be evaluated further. From the present study, it is observed that administration of NAC, curcumin as well as their co-administration might act as scavengers of the free radicals, thereby, preventing series of reactions responsible for lipid peroxidation of the cell membranes. The free radical scavenging activity of curcumin has been attributed to the hydrogen atom transfer or sequential and proton transfer from the two phenolic hydroxyl groups present in the molecular structure of curcumin. (Priyadarsini, 2013) NAC acts as a precursor for generation of GSH which metabolizes NAPQI to nonreactive conjugates, thereby, preventing the chain reactions for lipid peroxidation due to free radicals generated in the adduct formation by NAPQI. (Holdiness, 1991) Thus, NAC and curcumin have antioxidant actions due to different mechanisms and may have shown a hepatoprotective effect when co-administered together in acetaminophen induced hepatotoxicity.

Curcumin and NAC increased the acetaminophen induced depletion of liver GSH content in the present study. Increase in liver GSH content by curcumin in acetaminophen induced

hepatotoxicity in the previous studies was observed by Farghaly *et al*, Yousef *et al*, and Somanawat *et al*. (Somanawat *et al*., 2013; Yousef *et al*., 2010; Farghaly and Hussein, 2010) Co-administration of curcumin and NAC, when given by same as well as different routes, in half doses of each, significantly increased GSH content of the liver tissue compared to that of NAC used alone but GSH content was comparable when curcumin was used alone. Full doses of curcumin and NAC when co-administered together, the GSH levels of liver were comparable to each of the drug used alone. In the present study, it was observed that NAC, curcumin as well as their co-administration elevated the GSH content in the liver in acetaminophen induced hepatotoxicity. Hepatotoxicity induced by acetaminophen lowers the GSH levels in the liver which are not recovered to the original state till 48 hours of induction. (Ben-Shachar *et al*., 2012) The mechanism by which curcumin increases the GSH content has not yet been elucidated. (Farghaly and Hussein, 2010) However, curcumin has been observed to deplete the GSH content as curcumin is metabolized by glutathione conjugation, and thereby consumes GSH. (Priyadarsini, 2013) Hence, the action of curcumin as a scavenging agent of free radicals appears to be superior over its direct action on GSH content in the liver in presence of metabolites of acetaminophen. NAC provides sulfhydryl groups for regeneration of reduced glutathione from the oxidized glutathione and increases the rate of generation of NAC. (Ben-Shachar *et al*., 2012; Holdiness, 1991) The actions of NAC and curcumin in increasing GSH content occurs by different mechanisms and, hence, may have shown synergistic effect in comparison to NAC or curcumin used alone in acetaminophen induced hepatotoxicity.

NAC, curcumin and their co-administration failed to preserve the normal histology of the liver as observed with the mild necrotic focal lesions with acetaminophen. Improvement of histopathology of liver tissue by curcumin was observed in the previous studies by Kalantari *et al*, Yousef *et al*, Li *et al*, Kherapezhouh *et al*, Somanawat *et al*, Sayed *et al*, and Soliman *et al*. (Somanawat *et al*., 2013; Kheradpezhouh *et al*., 2010; Yousef *et al*., 2010; Soliman *et al*., 2014; Kalantari *et al*., 2007; Sayed and El-Kordy, 2014) Kheradpezhouh *et al*. observed improved histopathology of liver with NAC, curcumin as well as co-administration group. (Kheradpezhouh *et al*., 2010) The disagreement with previous studies regarding histology can be due to use of higher (2000 mg/kg) dose of acetaminophen compared to previous studies, causing more hepatotoxicity.

Curcumin and its co-administration with NAC prevented the increase in liver weight and volume due to acetaminophen toxic dose. Increase in liver weight in acetaminophen induced hepatotoxicity is observed in the initial few hours of induction due to increase in liver congestion which resolves with time. (Hinson *et al*., 2010) Liver weight and volume is not a reliable marker of hepatoprotection and concluding an effective hepatoprotectant on the basis of reduction in liver weight and volume is not possible. Increase in the liver weight and volume has been reported in acetaminophen induced hepatotoxicity models in initial 24 hours, following which it starts decreasing. (Hinson *et al*., 2010) Curcumin and the co-administration of curcumin and NAC might reduce the congestion in liver compared to NAC, as observed in the present study.

Conclusion

Co-administration of N-acetyl cysteine and curcumin in half doses of each drug, in acetaminophen induced hepatotoxicity in Wistar rats, has a hepatoprotective effect compared to N-acetyl cysteine or curcumin used in alone in full doses. This effect of N-acetyl cysteine, curcumin and its co-administration is due to antioxidant actions of each of the drug having different mechanisms. The hepatoprotective effect was reflected as reduction of transaminase enzymes and malondialdehyde levels in the liver and increase in the reduced glutathione content of the liver tissue.

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