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Piperine and Quercetin Enhances Antioxidant and Hepatoprotective effect of Curcumin in Paracetamol Induced Oxidative Stress

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Abstract: Drug induced hepatotoxicity is a category of physiological oxidative stress caused by 50% drugs. Curcumin obtained from *Curcuma longa* is a potent antioxidant and hepatoprotective but has low bioavailability. Piperine and Quercetin were combined with Curcumin enhances oral bioavailability by inhibiting metabolic enzyme. The enhanced availability was hypothesized to potentiate hepatoprotective activity of curcumin by enhancing the antioxidant activity. The *in vitro* and *ex-vivo* antioxidant activity was measured by DPPH and TBARS method, respectively. The albino wistar rats used for *in vivo* method were pre-administered for 7 days with curcumin combination consisting of Curcumin with Piperine and Quercetin (CPQ), silymarin was used as reference drug. The animals were challenged with paracetamol on 7th day to induce hepatotoxicity. Hepatoprotective activity was assessed by the levels of marker enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in plasma. The present findings of *in vitro* and *ex vivo* study showed statistical significance ($p<0.001$) in IC_{50} results with 50% enhancement of activity by combinatorial extract. The serum levels of ALT, AST and ALP were significant ($p<0.001$) decrease by 53%, 35% and 33%, respectively after treatment with combinatorial extract. The study also suggests that oxidative stress appears to play a major role in hepatic toxicity and administration of combination consisting of CPQ protects against paracetamol toxicity thus could be taken as a platform for further studies as dose of CPQ can be reduced when curcumin was given in combination with piperine and quercetin than curcumin alone.

Key words: Antioxidant, curcumin, hepatoprotective, piperine, quercetin

INTRODUCTION

Hepatotoxicity is universally defined as toxicity of liver via chemicals these are also known as hepatotoxins. The most common cause of hepatotoxicity is oxidative stress; drugs induced toxicity and xenobiotics. More than 900 drugs and there combinations have implicated evidences for liver injury due to their toxic effects. Approximately 20,000 deaths caused by liver disorder found every year (Rani *et al*., 2010; Naik and Panda, 2008). The chief reason for recurrent occurrence of hepatotoxicity is because liver comes in direct contact with drug/toxin concentrated blood from gastrointestinal tract thus making it most susceptible. Drug/toxin taken or generated in the body increase the levels of oxidants (reactive oxygen species) which cannot be curbed by non-enzymatic scavengers (antioxidants) as well as enzymatic systems (e.g., glutathione conjugation) are involved in the detoxification of reactive oxygen species. Oxidative stress is thus results from an imbalance between oxidants and antioxidants in favor of the oxidants. If oxygen and its metabolites are not evenly distributed in organs then such site are considered under physiological oxidative stress (Keaney, 1999).

The management of liver disease is still challenge to modern medicine. No drug has been developed in modern system of medicine which may stimulate the liver function, protect it from damage or helps in regeneration of hepatic cells. In the absence of a reliable liver protective drug and severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for traditional herbal medicines that are claimed to possess hepatoprotective activity (Kiritikar and Basu, 1996; Jain *et al*., 2007). Curcumin obtained from *Curcuma longa* or turmeric is a member of Zingiberaceae family which is a perennial herb with short and thick rhizomes. This herb has enormous potential for a variety of diseases including hepatotoxicity, higher safety margin than the synthetic drugs and is cost effectiveness (Wu *et al*., 2008;

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Vermeulen *et al*., 1992; Motterlini *et al*., 2000) Despite having wide spectrum of pharmacological actions, the medicinal properties of curcumin cannot be utilized due to its low *in vivo* bioavailability because of its highly lipophilic nature (Hegge *et al*., 2008). Therefore there is an extensive need for combinatorial extract which may enhance bioavailability of oral curcumin by inhibiting the enzymes responsible for the metabolism of curcumin. Piperine obtained from *Piper nigrum* inhibits enzyme gluconosyltransferase of curcumin metabolism and Quercetin (Guzy *et al*., 2004) obtained from *Allium cepa* inhibits the enzyme sulfotranferase. Thus to achieve enhanced effect of curcumin a combinatorial extract was prepared consisting of "Curcumin with Piperine and Quercetin (CPQ)" which may enhance bioavailability of oral curcumin.

Paracetamol an analgesic and antipyretic drug is extensively used and, though safe when used at therapeutic doses, is associated with significant hepatotoxicity when taken in overdose. Paracetamol on metabolism forms intermediate N-Acetyl-P-benzo Quinoneimine (NAPQI) which is toxic and is detoxified by glutathione (GSH). In situations of paracetamol overdose, sulfation and glucuronidation reaction process becomes highly active forming a large quantity of NAPQI. Thus this high level of toxic principles cannot be detoxified by GSH completely and the excess of NAPQI causes oxidative stress and binds covalently to liver proteins leading to liver cells death (Ojo *et al*., 2006; Manokaran *et al*., 2008; Somchit *et al*., 2005).

The study discussed in this study was taken with a view that combination of curcumin with Piperine and Quercetin extract (CPQ) has not been prior tested for the hypothesis of enhanced antioxidant and hepatoprotective effect in paracetamol induced oxidative stress over curcumin extract alone by *in vivo* method. The enhanced hepatoprotective effect of CPQ was estimated by the level of hepatic enzymes alamine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) which act as markers for oxidation in liver.

MATERIAL AND METHODS

Plant material: Dried rhizomes of *Curcuma longa* (Zingiberaceae) and dried seeds of *Piper nigrum* (Piperaceae) and the red onion of *Allium cepa* (Alliaceae) were collected from the local market of Mumbai, India and authenticated by Department of Raw and Crude drug material, National Institute of Science Communication and Information Resource (NISCAIR), New Delhi. The standardization of the procured plant material was done by examining the quality and purity of the procured material. The detailed pharmacognostical study with respect to morphology, microscopy and powder characteristics of *Curcuma longa* rhizomes, fruits of *Piper nigrum, Allium cepa* bulbs was carried out.

Extraction of plant materials: Curcuminoid and Piperine were isolated from *Curcuma longa* Linn and *Piper nigrum* in 95% ethanol. The alcoholic extract of Curcuminoid was concentrated to a semisolid brown colored mass and was then recrystallized by acetone. Whereas the concentrated pepper extract was added to of KOH solution and was heated in water till a yellow precipitate forms. The precipitate was recrystallized by acetone. Quercetin was extracted from *Allium cepa* by macerating in ethyl acetate followed by concentrating and recrystallization in acetone.

Combinatorial extract of curcumin was prepared by suspending curcumin, piperine and quercetin in a ratio of 94:1:5, respectively in 5% Gum Acacia and 0.5% Tween 80.

Animals: Swiss albino mice of both sexes were used in acute toxicity investigation and hepatotoxicity was induced in Wister rats weighing 150-300 g of either sex using paracetamol (2 g kg^{-1} body weight). Experimental protocol number CPCSCA/SPTM/P-82/2009 was reviewed and approved by the Institutional Animal Ethics Committee. The animals were maintained in polypropylene cage in the Departmental Animal House Facility with 12 h light:12 h dark cycle. All the animals were kept under laboratory condition (temperature 25±2°C; relative humidity 75%±5%) for an acclimatization period of 7 days before carrying out the experiments. During the experiments animals were provided with standard rodent pellet diet (Amrut laboratory animal feed, Maharashtra) and filtered water was provided *ad libitum*. The experiment was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experimentation on Animals, India and was approved by Animal ethical committee, Department of Pharmacology, School of Pharmacy and Technology Management, Narsee Monjee Institute of Management and studies, Mumbai.

Acute toxicity study: Mice were randomly divided into three groups, each containing six animals .The combinatorial extract was administered orally at doses of 500, 1000 and 2,000 mg kg^{-1} of body weight (OECD, 423). Distilled water was administered to control group. The general behavior of the mice was continuously monitored for 1 h after dosing, periodically during the first 24 h with

special attention given during the first 4 h and daily thereafter, for a total of 14 days. The changes in the normal activity of mice and their body weights were monitored and the time at which signs of toxicity or death appeared recorded.

Chemical: DPPH was procured from Sigma Aldrich and enzymes ALT, AST and ALP working standards were procured as a part of Erba Diagnostic Kit, manufactured by Trans Biomedical LTD. All other chemicals along with Paracetamol were procured from SD Fine chemicals, India.

Preliminary phytochemical screening: The alcoholic extract of curcumin, piperine and quercetin were taken for various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils, protein and amino acids, flavanoids, saponins, gums and mucilage (Harborne, 1998). The compounds were further identified using chromatographic techniques against reference standards.

Determination of DPPH radical scavenging activity (Antolovich *et al.*, 2002; Ulyana *et al.*, 2002): A rapid, simple, and comparatively inexpensive method to measure antioxidant activity is based on the reduction of methanolic solution of colored free radical 1, - Diphenyl-2- picryl hydrazyl DPPH (0.36 mg mL^{-1}) by free radical scavenger. Ascorbic acid was used as a standard and linearity was established by varying the volume of standard and test stock solution (0.25 mg mL^{-1}) to get concentration in range of 1-32 mcg mL^{-1}. 200 µL of DPPH solution was added to each solution to make final volume of 3 mL with methanol. Absorbance was taken after 15 min at 516 nm using methanol as blank on Perkin Elmer UV-Vis spectrophotometer Lambda 25. The IC_{50} values for curcumin and combinatorial extract were then calculated and compared with value of Ascorbic acid taken as a positive control.

Determination of lipid peroxidation inhibitory activity by TBARS method: Antilipid peroxidation abilities of Combination consisting of CPQ over curcumin extract was evaluated by Thiobarbituric Acid Reacting Substances (TBARS) method (Ravishankara *et al.*, 2002; Bergamini *et al.*, 2004). To measure the adduct of Malondialdehyde with Thiobarbituric acid rat was sacrificed using anesthetic ether, liver was quickly removed and chilled in ice cold saline and homogenized in 0.15 M KCl to get 10% liver homogenate. The liver homogenate was mixed with 0.15 M KCl and Tris buffer followed by addition of various concentrations of CPQ and curcumin extracts (1-32 mcg mL^{-1}). *In vitro* lipid peroxidation was initiated by addition of ferrous sulphate (10 µM) and ascorbic acid (100 µM). After incubation for 1 h at 37°C, reaction was terminated by addition of Thiobarbituric acid reagent (2 mL) and boiled for 15 min for development of colored complex; sample was centrifuged, cooled and then estimated spectrophotometrically at 532 nm. The inhibition of lipid peroxidation was determined by calculating the% reduction in formation of TBARS and expressed in terms of IC_{50}.

Induction of hepatotoxicity using paracetamol: The hepatoprotective effect was tested on five groups of Wistar rats, each group consisting of six animals. The first group (I) acted as negative control and were administered only the vehicle of 0.5% CMC (2 mL kg^{-1} orally) for 7 days. The second group (II) received vehicle for 7 days and on 7th day they received Paracetamol (2 g kg^{-1} orally) suspended in 0.5% CMC. Group three, four and five (III, IV and V) received standard silymarin (25 mg kg^{-1} orally), curcumin and combination consisting of CPQ (100 mg kg^{-1} orally), respectively suspended in 5% Gum Acacia and 0.5% Tween 80, once daily for 7 days and single dose of paracetamol (2 g kg^{-1}, orally) suspended in 0.5% CMC was given on the 7th day after 30 min after the last dose of extract.

At the beginning and at end of experimental period, all the animals were anesthetized and blood was withdrawn from the retro-orbital route. Blood samples were collected, using heparin as anti-coagulant. Plasma was separated by centrifuging at 4000 rpm for 10 min stored at -80°C prior to analysis.

Estimation of ALT (Alanine amino transferase), aspartate aminotransferase (AST) and alkaline phosphatase (ALP): The biochemical parameters like ALT, AST and ALP were estimated using ERBA Chem-7 Trans Asia. The levels of ALT and AST were estimated using previously set protocol by taking 1 mL of working reagent and 100 µL of distilled water in blank and 100 µL of control and test plasma was added in to tubes and estimated. The level of ALP were also estimated by using 1000 µL of working reagent along with 20 µL of distilled water in blank and 20 µL of control and test plasma.

Statistical analysis: The data were expressed as mean±SD and statistically assessed by t test and one-way analysis of variance (ANOVA) followed by Bonferroni's test.

RESULTS

Extraction of plant materials: The percentage yield obtained from *Curcuma longa* Linn and *Piper nigrum*

Piperine, respectively. The % yield of Quercetin after extraction from *Allium cepa* was 0.1% w/w.

Preliminary phytochemical screening: The extracts of curcumin, piperine and quercetin when tested preliminary for its phytoconstituents revealed the presence of alkaloids, steroids, saponins, triterpenes, flavanoids and polyphenolic compounds.

Acute toxicity study: The combination consisting of CPQ was found to be non toxic up to the dose of 2 g kg^{-1} and did not cause any mortality or symptoms of toxicity. According to Organization for Economic Cooperation and Development (OECD, 423) guidelines for acute oral toxicity, An LD_{50} dose of 2000 mg kg^{-1} and above is categorized as "unclassified" and hence drug is found to be safe. So further dosing to find out LD_{50} of combinatorial extract of curcumin was not performed.

Table 1: DPPH free radical scavenging activity of ascorbic acid, curcumin alone and CPQ

Concentration ($\mu g\ mL^{-1}$)	Inhibition (%)		
	Ascorbic acid	Curcumin	Combinational extract
1	27.50±1.69	2.61±0.30	4.62±0.66
2	31.68±0.95	4.46±0.30	8.69±0.47
4	36.99±1.25	8.62±0.17	16.22±0.24
8	47.80±1.61	16.93±1.62	27.51±1.10
16	61.14±1.51	27.12±0.52	41.85±0.32
32	96.61±1.13	50.89±3.44	76.37±1.08
Linearity-R^2	0.9954	0.9951	0.9911
IC 50	8.667	31.67***###	17.00**

Values are as Mean±SD with n = 3. One-way ANOVA followed by Bonferroni's test was applied for statistical analysis, Extract treated groups were compared with standard treated, ***Significant at p<0.01, **Significant at p<0.001. Combinatorial extract treated group compared with curcumin alone, ###Significant at p<0.001

Table 2: Lipid peroxidation inhibitory activity of Curcumin alone and CPQ by TBARS method

Concentration ($\mu g\ mL^{-1}$)	Inhibition (%)	
	Curcumin	Combinational extract
1	20.35±0.76	26.56±1.16
2	23.56±0.87	33.39±1.19
4	30.34±1.04	38.97±0.19
8	36.91±0.49	49.93±1.62
16	51.41±0.45	70.69±1.05
32	75.50±1.61	98.17±1.91
Linearity-R^2	0.9892	0.9804
IC50	15.56***	7.2

Values are as Mean±SD with n = 3. T-test was applied for statistical analysis, Combinatorial extract treated group compared with curcumin alone, ***Significant at p<0.001

Determination of DPPH radical scavenging activity: Table 1 shows the result of DPPH free radical scavenging activity of curcumin and combinatorial extract. The 50% inhibition was obtained at concentration of 31.67 and 16 mcg mL^{-1} for curcumin and combinatorial extract, respectively. The statistical results by one way ANOVA test showed that average IC_{50} values for the combinatorial extract were significant different ($p<0.001$) to curcumin extract as shown in Table 1.

Determination of Lipid per oxidation inhibitory activity by TBARS method: The antioxidant activities of the combination consisting of CPQ and curcumin extract were compared by lipid peroxidation inhibitory activity using TBARS method the results were tabulated in Table 2. The average IC_{50} of Curcumin extract and combinatorial extract were obtained to be 15.56 $\mu g\ mL^{-1}$ and 7.2$\mu g\ mL^{-1}$, respectively. The t-test results showed that average IC_{50} values for the activity of combinatorial extract over curcumin alone were significant different ($p<0.001$) as shown in Table 2.

Estimation of ALT, AST and ALP: The serum levels of a number of hepatic lysosomal enzymes were used as diagnostic markers of hepatic injury. Increased levels of ALT, AST and ALP in serum of the Paracetamol treated animals indicate liver damage as these enzymes leak out from liver cells into blood at the instance of tissue damage. On concurrent treatment with curcumin and CPQ extracts as well as the reference drug Silymarin, the levels of these marker enzymes in serum were near normal or only slightly elevated, indicating protection against liver damage. The level of decrease in the serum levels of ALT, AST and ALP for curcumin extract is up to 31, 38.4 and 47.85%, respectively and decrease in the serum levels for combinatorial extract is up to 84, 74 and 81% , respectively (Table 3). Table 3 also shows statistical significance among the results obtained for levels of these hepatic enzymes. These decreases in the level may be due to the prevention of leakage of the intracellular enzyme by its membrane stabilizing activity. This in agreement with the commonly accepted view that serum levels of transaminase return to normal with regeneration of hepatocytes.

Table 3: Effect of CPQ on AST, ALT and ALP in paracetamol induced hepatotoxicity

Groups	AST(IU L^{-1})	ALT(IU L^{-1})	ALP(IU L^{-1})
Negative control (Saline sol. 2 mL kg^{-1})	103.03±4.41	49.33±12.05	165.80±17.65
Positive control (Paracetamol-2 g kg^{-1})	216.72±18.05	103.36±7.32	331.70±12.51
STD-Silymarin (25 mg kg^{-1})	125.15±7.24	60.54±6.24	186.13±8.96
Curcumin extract (100 mg kg^{-1})	184.22±3.94	84.40±3.19	252.35±14.52
Combinatorial extract (100 mg kg^{-1})	132.28±10.55	66.55±4.83	196.25±10.56

Values are as Mean±SD with n = 6

DISCUSSION

Approximately 2000 cases of acute liver failure are reported annually and drugs account for over 50% of them (39% are due to paracetamol, 13% are idiosyncratic reactions due to other medications). Drugs account for 2-5% of cases of patients hospitalized with jaundice and approximately 10% of all cases of acute hepatitis (Mehta and Pinsky, 2010). Oxidative stress, a major cause of hepatotoxicity is caused by excessive formation of reactive oxygen species which are byproducts of multiple reactions taking in our body (Kappus, 1987; Fridovich, 1998; Bergamini *et al*., 2004; Marks *et al*., 1996). The reactive free radicals overwhelm the protective enzymes causing destructive and lethal cellular effects by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration (Hazra *et al*., 2008; Bandyopadhyay *et al*., 1999). The oxidative imbalance and decrease in endogenous antioxidants leads to release of ALT (Alamine amino transferase), aspartate aminotransferase (AST) and alkaline phosphatase enzymes (ALP) (Rasool *et al*., 2007; Pimple *et al*., 2007). Since the reactive oxygen species play one of the major roles in hepatotoxicity it was considered to evaluate the effect of antioxidant on liver enzymes.

Curcumin is an herbal medicine used from ancient times in ayurvedha to cure array of disease conditions. It potency and efficacy of curcumin is particularly dependent on the rate of metabolism and solubility in the body fluids. The paper focuses on reduction of the rate of metabolism by use of piperine and quercetin. These herbal constituents have an ability to inhibit curcumin's metabolic enzymes thus may enhance its antioxidant and hepatoprotective activity. In this article hepatotoxicity was induced by an overdose of the analgesic/antipyretic Paracetamol. This overdose produces centrilobular hepatic necrosis (Mitchell *et al*., 1973a) through a critical step of cytochrome P450 metabolism of paracetamol to N-Acetyl-P-benzo Quinone Imine (NAPQI). NAPQI reacts with enzymatic antioxidant glutathione (GSH) (Jaeschke *et al*., 2002) leading to its depletion by as much as 90% (Mitchell *et al*., 1973b). The toxicity also can to related to formation of peroxynitrite, a highly reactive nitrating and oxidizing species which is formed by the rapid reaction of Nitric Oxide (NO) and superoxide, produces nitrated tyrosine correlates with necrosis. (Beckman, 1996; Pryor and Squadrito, 1995).

The efficacy of plant drug depends upon its quality and purity, thus making standardization at important assignment before checking its efficacy. A detailed pharmacognostical study with respect to morphology, microscopy and powder characteristics of *Curcuma longa* rhizomes, fruits *of Piper nigrum, Allium cepa* bulbs was carried out. The rhizome of *Curcuma longa* showed presence of benzene shaped cork cells, oleoresin cells, and wood elements whereas beaker-shaped stone cells and spiral vessel elements are identifying characters present in fruit of *Piper nigrum* and *Allium cepa* bulbs, respectively. Determination of physicochemical parameters like extractive values, ash value and loss on drying, foreign organic matter was carried out and were found to be within limits.

The isolated extract of curcumin, piperine and quercetin showed major presence of alkaloids and flavanoids. The combination consisting of Curcumin with Piperine and Quercetin (CPQ) were also evaluated for phytochemical screening and the result indicated the presence of flavanoids, carbohydrate, alkaloids, steroids, glycosides and phenolics compound. The identity of the compound was confirmed by comparison with those from a reference standard using Fourier Transform Infra Red (FTIR) and High-Performance Thin-Layer Chromatographic (HPTLC) method. The flavanoids in the isolated extract have been reported to have antioxidant activity and thus also justifying enhanced effect of the combinatorial extract as antioxidant (Kiritikar and Basu, 1996).

The acute toxicity studies showed the constituents in the extracts were found to be non lethal as no mortality was observed from 500 mg mL^{-1} to 2 g mL^{-1}. The concentration of up to 2 g mL^{-1} showed no toxic effect in all the animals taken in the test. Overall, this study provides valuable data on toxicity profile of combinatorial extract consisting of curcumin, piperine and quercetin that should be useful for the planning of future preclinical of the medicinal plant.

The *in vitro* testing of antioxidant activity was established using DPPH method. The extracts could inhibit the odd electron in DPPH free radical which shows a strong absorption band at 517 nm and its solution appears as deep violet color which changes to yellow, as this electron becomes paired with hydrogen from a free radical scavenging antioxidant. The IC_{50} value showed statistical significance ($p<0.001$) in results of the test of curcumin and CPQ extract were 31.67 and 17 mcg mL^{-1}, thus showing that CPQ is a better antioxidant as lower concentration gives same effect.

The *ex vivo* testing of antioxidant was established using TBARS method. In this method the extracts measures the malondialdehyde formed form oxidation of lipid substrate isolated microsomes from rat liver are induced with ferric ions. Thus free radicals are generated by ferrous-ascorbate system. Malondialdehyde forms a 1:2 adduct with thiobarbituric acid in acidic condition at

80°C producing pink colored complex, which was measured at 532 nm. The test results showed IC_{50} values of curcumin at 15.56 mcg mL^{-1} and combinatorial extract at 7.2 mcg mL^{-1}, thus both were found to significantly ($p<0.001$) inhibit ferrous sulphate induced lipid peroxidation in rat liver homogenate. This anti-lipid peroxidation activity it may be concluded that these drug effective as a hepatoprotective. The *in vitro* and *ex vivo* enhance activity of CPQ over curcumin to about 50% shows that the drug dose can be reduced when given in combination than curcumin alone.

The antioxidant activity of curcumin and combinatorial extract when further tested for their effects on paracetamol induce acute oxidative stress in liver. Smaller doses of paracetamol get eliminated by conjugation followed by excretion, but when the conjugating enzymes get saturated, the drug is diverted to an alternative metabolic pathway, resulting in the formation of a hydroxylamine derivative by cytochrome P_{450} enzyme and thus probably leading to toxicity. The extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Induction of cytochrome P_{450} or depletion of hepatic glutathione is a prerequisite for parac etamol-induced toxicity (James *et al*., 2003; Rao *et al*., 2006).

The extracts were thus hypothesized to act as antioxidants by either inhibit the formation of the toxic paracetamol metabolite or stimulate the hepatic regeneration (Rao *et al*., 2006). The initial pretreatment with the extracts for 7 days is assumed to stimulate the liver to become more resistant to damage by toxins. Paracetamol intoxication in normal rats elevates the levels of AST, ALP and ALT significantly, indicating acute centrilobular necrosis. These levels when tested after treatment showed combinatorial extract more effective then the curcumin extract and this was statically significant. The enhanced *in vivo* activity of CPQ over curcumin to about 30% shows that the drug dose can be reduced when given in combination than curcumin alone. Thus from these *in vivo* results of AST, ALT and ALP enzymes it can be further concluded that the antioxidant activity of both the extract helps to enhance hepatoprotective effect of curcumin by inhibiting the free radical formed and preventing lipid peroxidation in liver.

CONCLUSION

The present study results indicates that the combination consisting of curcumin with piperine and quercetin is safe, nontoxic and is more effective in inhibiting DPPH radicals, malondialdehyde formed in TBARS method and the enzyme AST, ALT and ALP than curcumin extract alone. Enhanced activity could be because of piperine and quercetin contribution to decrease curcumin metabolism by inhibiting enzymes and thus confirming the set hypothesis. The study thus provided more insight into the mechanism of the hepatoprotective action of combinatorial extract consisting of CPQ and also provides a scientific basis for its usage in the traditional systems of medicine, for the management of hepatotoxicity.

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