Dose-Response:
An International Journal
January-March 2021:1-9

DOI: 10.1177/1559325821996955
journals.sagepub.com/home/dos
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In Vitro Antioxidant Activities and the Therapeutic Potential of Some Newly Synthesized Chalcones Against 4-Acetaminophenol Induced Hepatotoxicity in Rats

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Abstract

The lack of safety and efficacy of existing hepatoprotective agents urge the need to explore novel hepatoprotective agents. The research work was planned to study the therapeutic potential of some newly synthesized chalcones against 4-acetaminophenol induced hepatotoxicity in rats. Male albino rats (N = 30) were divided into 6 groups of 5 animals each i.e. group I; Toxic control (4-acetaminophenol), group II; normal control (Normal saline), group III; Positive control (silymarin; 50 mg/kg bw) and groups IV-VI (test groups) treated with 3 chalcone analogues i-e 3a, 3f & 3 g (100, 150, 150 mg/kg bw, respectively). All the study group animals were administered with 4-acetaminophenol to induce hepatotoxicity except normal control. Following hepatotoxicity induction, test group animals were administered with selected doses of test compounds and toxic group animals left untreated. Liver enzymes including ALT, AST, ALP and serum bilirubin were determined photometrically. Antioxidant activities of test compounds were also determined. Histopathological examination of liver biopsies was also carried out through H & E staining. The test chalcones (**3a**, **3f** & **3 g**) significantly decreased the levels of liver enzymes and serum bilirubin toward normal and the pattern of results in the test group animals were comparable to silymarin administered animals indicating the hepatoprotective potential of test compounds. Moreover, the test chalcones (**3a**, **3f** & **3 g**) antagonized the effect of 4-acetaminophenol and thus, raised the catalase (CAT) and superoxide dismutase (SOD) while decreased the malondialdehyde (MDA) in experimental animals. The test chalcones (**3a**, **3f** & **3 g**) on histological examination of liver showed improvement of tissue morphology. The study

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Received 24 December 2020; received revised 23 January 2021; accepted 25 January 2021

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concluded that the tested compounds have antioxidant potential and may act as hepatoprotective agent. However, in-depth studies are required to validate their safety and to elucidate the exact mechanism of action.

Keywords
hepatoprotective, antioxidant, chalcones, 4-acetaminophenol, liver enzymes, histology

Introduction

Liver is the vital organ of the body that plays a key role in the maintenance of homeostasis and detoxification of xenobiotics.[1] Structurally liver consists of hepatocytes, endothelial cells and kupffer cells.[2] Various biochemical reactions such as building up the complex molecules are regulated by liver enzymes which also induces oxidative stress because of reactive oxygen species (ROS) generation. Detoxification of noxious chemicals involves the conversion of ROS into non-toxic compounds by antioxidants (glutathione & tocopherol).[3] Antioxidants slow or prevent the oxidative injury by minimizing the oxidation of substrates either by inhibition of free radical formation or the propagation step or by chelation of metal ions.[4] The 4-acetaminophenol (*N*-acetyl-*p*-aminophenol)-induced hepatotoxicity remains a global challenge and in particular in the United States, it accounts for more than 50% of overdose-related acute liver failure and approximately 20% of the liver transplants.[5] PCM induced hepatic toxicity is dose dependent and involves apoptosis, necrosis or disruption of hepatic cells.[6] It is associated with increased serum levels of enzymes, i.e. Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) and total bilirubin levels. Immunosuppressant and corticosteroids used in the treatment of hepatic disorders, which have adverse effects like osteoporosis, hypertension, weight gain and eye disorders etc. Thus, for liver diseases successful management is still awaited and hepatic disorders are a global health challenge.[7] The activity of antioxidant enzymes including CAT and SOD is reported to be decreased in PCM-induced liver injury. Moreover, lipid peroxidation leads to hepatic damage that can be observed by increased serum and tissue homogenate levels of MDA.[6] Redox homeostasis in hepatic tissues shifts toward ROS generation with marked decrease in antioxidant capacity. DPPH assay can be used for *in-vitro* assessment of antioxidant activities of various anti-oxidants.[8] Hepatic necrosis and mitochondrial damage can be seen through histological examination of liver tissues from animals suffered from 4-acetaminophenol-induced hepatotoxicity.[5]

There are number of herbal and synthetic agents that are reported to be used in the management of liver diseases among which medicinal plant commonly known as milk thistle or silymarin (*Silybum marianum*),[9] which is composed of mainly flavonoids (silibinin, silichristin, silidianin and isosilibinin) with antioxidant and free radical scavenging properties.[10] Synthetic compounds like curcumin analogue, 1,7-diphenylhepta-1,6-diene-3,5-dione (DDD), tempol and MnSODm (manganese superoxide dismutase) have shown hepatoprotective effects in various experimental models of drug induced hepatic insult.[11] Chemically, chalcones are flavonoids in open chain form with 2 aromatic rings associated *via* a 3 carbon α,β-unsaturated enone system and are found widely in numerous plant species. These compounds are used in traditional medicinal system to treat variety of diseases. Whether extracted from plants or of synthetic origin, chalcones have been found associated with diverse biological applications (such as antioxidant, antipyretic, cytotoxic, anti-inflammatory, antitumor and anti-mutagenic etc.) due to their characteristic conjugated molecular architecture.[12] Naturally occurring chalcones derived from general foods are phloretin and its glucoside phloridzin, chalconaringenin and arbutin. In context of synthetic approaches of chalcones, a number of studies focused on the synthesis of the 1,4-enones using acid- or base-catalyzed condensation reactions of aldehyde and aryl methyl ketones.[13] Notably, curcumin and its related enones, inhibited the activation of NF-kβ and up-regulation of COX-2. The hepatoprotective effects of chalcone derivatives have also been reported in D-galactosamine/lipopolysaccharide (D-GalN/LPS)-induced fulminant hepatic failure in mouse model. The chalcones possessed dual antioxidant mechanisms and therefore could be very promising as anti-ischemic stroke agents.[14] Moreover, various chalcone analogues have also shown beneficial effects in animal models of diabetes.[12]

To the best of our knowledge, antioxidant, anti-inflammatory, antibacterial and antiviral effects of chalcone analogues are well documented however; hepatoprotective effects have not been studied in 4-acetaminophenol-induced hepatic damage so far. Moreover, the chalcones are flavonoids which are hepatoprotective in nature.[10] Therefore, we aimed to study the hepatoprotective and antioxidant activities of newly synthesized chalcones (**3a-g**) in PCM-induced model of hepatotoxicity in experimental rats. Newly synthesized 7 chalcones (**3a-g**) were evaluated for their hepatoprotective activity in the preliminary screening study followed by detailed testing (liver markers, antioxidant activities and liver histology) with only 3 selected novel compounds.

Material and Methods

Chalcones 3a-g

Seven different chalcones were prepared following the reported method already reported by our research group.[15] The method was based upon H_2SO_4 catalyzed condensation reaction of a ketone **1a-c** and an aldehyde **2a-e** to form chalcones **3a-g**. The ketone **1a-c** (7.5 mmol, 1 eq.) and aldehyde **2a-e** (7.9 mmol, 1.05 eq.) were ground together mechanically with grinder in the presence of solid NaOH (0.30 g, 7.60 mmol, 1.01 eq) for

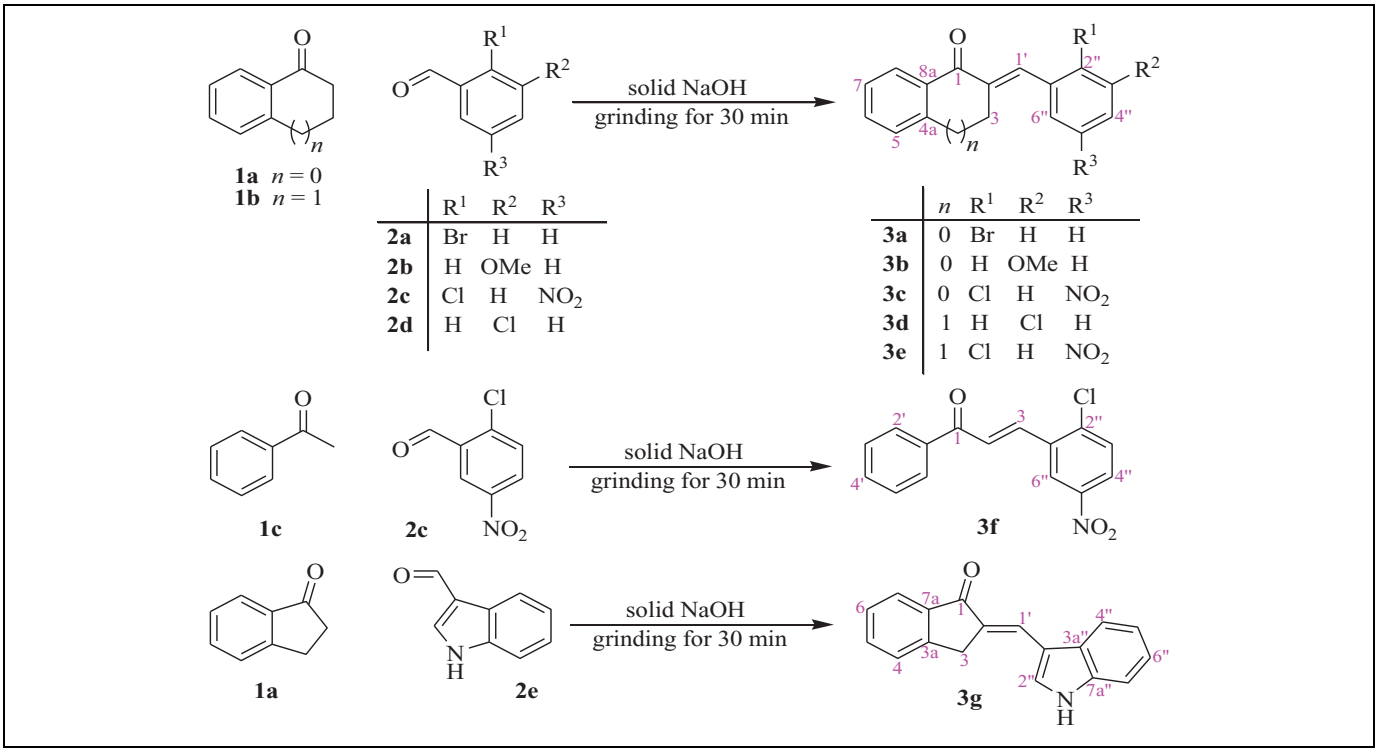

Figure 1. The synthesis of chalcones **3a-g**.

30 min. The reaction mixture was extracted with Et_2O (3 ×10 mL) after neutralization. The organic layer was dried over Na_2SO_4 (anhydrous), filtered and concentrated under reduced pressure for the affordability of the desired product as colorless solid (Figure 1). The synthesized chalcones were characterized using IR, ^{1}H-NMR, ^{13}C-NMR and EIMS techniques before their biological evaluation. The spectroscopic and spectrometric data of most potent compound (**3f**) is reproduced.

(*E*)-3-(2-Chloro-5-nitrophenyl)-1-phenylprop-2-en-1-one (**3f**): Off-white crystalline solid (85%); Rf: 0.59 (EtOAc/n-hexane, 1:1); M.p.: 173°C; IR (KBr) ύ (cm–1): 1676 (C = O), 1512 (N = O), 682 (C–Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ in ppm): d (ppm) 7.58 (3 H, t, J = 7.2 Hz, H-3″, H-4″), 7.66–7.69 (2 H, m, H-2″), 7.68 (1 H, d, J = 15.9 Hz, H-2), 8.10 (1 H, d, J = 7.2 Hz, H-3′), 8.19 (1 H, d, J = 15.9 Hz, H-3), 8.23 (1 H, dd, J = 7.2, 2.4 Hz, H-4′), 8.65 (1 H, d, J = 2.4 Hz, H-6′); 13C-NMR (75 MHz, CDCl3, δ in ppm): 122.6 (C-3′), 125.1 (C-4″), 127.0 (C-2), 128.7 (2, C-2″ or C-3″), 128.9 (2, C-2″ or C-3″), 131.3 (C-3), 132.0 (C-1″), 133.5 (C-3), 134.9 (C-1′), 137.0 (C-2′), 138.0 (C-6′), 146.8 (C-5′), 189.4 (C-1); EI MS (m/z): 287, 289 $[M]^+$ (23, 7%), 105 $[PhCO]^+$ (78%), 77 $[Ph]^+$ (100%); ESI HRMS (amu): 310.0954, 312.0926 $[M + Na]^+$ (found in 3:1) for 310.0247, 312.0217.

Chemicals and Equipments

All chemicals (**1a-c**, **2a-e**, Et_2O, anhydrous Na_2SO_4, etc.) used in the study were of analytical grade (Merck / Sigma / Aldrich / Fluka). The carboxymethylcellulose (CMC) (Merck Chemical Co., Germany, CAS 9004-32-4), 4-acetaminophenol (Pacific Pharmaceuticals Ltd, Lahore), silymarin (source, *Silybum marianum*) (Abbott Laboratories, Pvt. Ltd. Karachi) was used. The pre-coated silica gel (0.25 mm thick layer over Al sheet, Merck) TLC was used to monitor the reaction. The IR and UV/Vis spectra were recorded on Shimadzu (Prestige 21) FT-IR spectrometer as KBr discs and Thermo Spectronic (UV-1700) spectrophotometer in CHCl3/MeOH, respectively. The ^{1}H-NMR and ^{13}C-NMR were recorded in $CDCl_3$ on a Bruker AVANCE DPX300/400/500 (300, 400 or 500 MHz) spectrometer using Me4Si as internal standard. The LR ESI was recorded on Q-TOF Ultima API (Micromass) at the Biomedical Mass Spectrometry Facility (BMSF), UNSW, Sydney (Australia). The diagnostic kits used for ALP, AST, ALT and bilirubin measurements were purchased from Diasys Diagnostic System GmbH, Germany. The Ultra-violet Visible Spectrophotometer (Shimadzu Corporation Kyoto, Japan) was used for recording absorbance. All the chemicals (thiobarbituric acid (TBARS), tris-cacodylate, DPPH etc.) used in MDA, SOD, CAT and DPPH assays were of analytical grade.

Experimental Animals

Adult healthy male albino rats weighing 150-200 g were procured from University of Agriculture, Faisalabad-Pakistan. The animals were housed in stainless steel cages at the animal house, College of Pharmacy, University of Sargodha under standard conditions (temp. 24 ± 2°C, humidity 45-55% and 12 h light/dark cycle) and fed with standard rat diet and water *ad libitum*. For acclimatization, animals were kept in the cage

Table 1. Effects of 7 Synthetic Chalcones (**3a-g**) and 3 Selected Test Chalcones (**3a**, **3f** and **3 g**) on Liver Function Markers.

	Treatment groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dL)
Preliminary experiment	Disease control (4-acetaminophenol 2g/kg)	189 ± 25.249	275.5 ± 3.0	549.5 ± 12.9	1.225 ± 0.07
	Normal control (normal saline 10 mL/kg)	54.5 ± 3.7	172.5 ± 4.5	423.2 ± 16.6	0.55 ± 0.29
	Reference drug (Silymarin 50 mg/kg)	79 ± 1.47*	190 ± 3.85*	428 ± 7.22*	0.61 ± 0.01*
	3a (100 mg/kg)	86.2 ± 3.79*	241.5 ± 2.36*	474 ± 14.77*	0.96 ± 0.05*
	3b (100 mg/kg)	193.2 ± 45.25^{ns}	276.5 ± 9.25^{ns}	523 ± 8.63^{ns}	1.2 ± 0.04^{ns}
	3c (100 mg/kg)	110.2 ± 3.88*	249 ± 9.79*	489.5 ± 7.66*	1.01 ± 0.05*
	3d (100 mg/kg)	122 ± 3.89^{ns}	263.25 ± 4.32^{ns}	512.5 ± 5.17^{ns}	1.12 ± 0.02^{ns}
	3e (100 mg/kg)	120.5 ± 4.5*	250.5 ± 6.45*	493.2 ± 4.58^{ns}	1.01 ± 0.05*
	3f (50 mg/kg)	63 ± 4.6*	241.5 ± 2.36*	321.25 ± 30.84*	0.95 ± 0.03*
	3 g (100 mg/kg)	61.25 ± 2.86*	243.25 ± 3.11*	477.5 ± 14.26**	0.97 ± 0.04**
Validation experiment	**3a** (150 mg/kg)	65 ± 2.55*	182 ± 2.27*	400 ± 10.68*	0.60 ± 0.02*
	3f (100 mg/kg)	38.25 ± 3.17*	158.5 ± 3.12*	316.25 ± 30.84*	0.48 ± 0.03*
	3 g (50 mg/kg)	57.25 ± 2.05*	166.5 ± 2.78*	400.25 ± 11.6*	0.55 ± 0.02*

Enzymes are expressed as Unit per Liter (U/L) and Bilirubin as Milligram Per Deciliter (mg/dL) while values are statistically presented as Mean ± SEM, n = 5; One way ANOVA followed by Dunnett's "t" multiple comparison test was used; * = $P \leq 0.05$ (significant).

for 7 days before starting the experiment.[16] All protocols were conducted in the College of Pharmacy according to the agreement of the Institutional Animal Ethics Committee, University of Sargodha, Sargodha, Pakistan (1169/ac/08/CPCSEA).

Experimental Procedures

Administration of drugs to animals. The suspensions of reference drug silymarin, 7 test compounds and 4-acetaminophenol were freshly prepared in aqueous solution of 0.5% CMC daily. The test compound doses were randomly selected in accordance with the dose of standard drug silymarin. Oral gavage, using 1 cc B.D syringe connected with a special oral needle, was performed for administration of drug suspensions including test compounds, reference drug and 4-acetaminophenol directly into animal's stomach.

Preliminary experiment. Preliminary experiment was performed for initial screening of a series of 7 chalcone analogues for hepatoprotective activity against 4-acetaminophenol-induced hepatic toxicity.[17] Animals were divided into 10 groups (n = 5/group) and received following treatments orally: Group I (Disease control); normal saline (NS) daily and 4-acetaminophenol 2g/kg on day 7, Group II (Normal control); NS daily, Group III; silymarin (50 mg/kg) daily and 4-acetaminophenol on day 7, Group IV; test compounds **3f** (50 mg/kg) and 4-acetaminophenol on day 7 and Group V-X; test compounds daily [**3a-e** and **3 g** (100 mg/kg) respectively] and 4-acetaminophenol on day 7. The test compounds were administered daily for 7 days orally and 4-acetaminophenol single dose at dose rate of 2 g/kg on 7th day at lag time of 2 h after last test compound dose. The liver markers including ALP, AST, ALT and total bilirubin were determined on 8th day. The levels of AST, ALT, ALP and total bilirubin were found to be decreased significantly in animals administered with test compounds namely: **3a**, **3f** and **3 g** (Table 1). These compounds were selected for detailed studies in higher doses (100, 150, 150 mg/kg).

Hepatoprotective Potential Against 4-Acetaminophenol-Induced Hepatotoxicity

Animals were divided into 6 groups (n = 5/group) and received following treatments: Group I-III; same as described in preliminary experiment, Group IV; test compound **3f** (100 mg/kg) daily and 4-acetaminophenol on day 7 and Group V-VI; test compounds daily [**3a** and **3 g** (150 mg/kg)] and 4-acetaminophenol on day 7. On the 8th day, rats were weighed, euthanized, dissected (using ether for anesthesia) and blood samples were collected by cardiac puncture. Then sera were obtained through centrifugation and preserved at −20 °C for subsequent analysis. The liver markers (ALT, AST, ALP and total bilirubin) were determined by using standard commercial kits measured through UV spectrophotometer.

Antioxidant Activities of Chalcone Analogues

In-vivo antioxidant activities were determined by 3 different methods: Malondialdehyde (MDA),[18] Catalase (CAT)[19] and Superoxide Dismutase (SOD)[20] while *in vitro* anti-oxidant activity was measured through 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method.[21]

Histological Examination of Liver

For histological examination, animals were euthanized with ether anesthesia; liver tissue of animals were excised, washed with phosphate buffer and transferred into formalin (10%) solution. Liver tissue were then embedded in paraffin, cut into thick (4-5 μm) sections using microtome, stained (haematoxylin & eosin dyes) and microscopically observed for morphological alteration in PCM and treatment groups.[22]

Table 2. Effects of 3 Synthetic Chalcones (**3a**, **3f** and **3 g**) on Body Mass Gain in Rats.

Treatment groups	Body mass (g)		
	Before treatment	After treatment	% change in body mass
Disease control (4-acetaminophenol 2g/kg)	169 ± 4.89	143 ± 4.71	−14.75 ± 0.25
Normal control (normal saline 10 mL/kg)	151.5 ± 1.44	160.75 ± 1.11	5.5 ± 0.29
Silymarin (50 mg/kg)	170.25 ± 5.28	178 ± 5.34	4.0 ± 0.91
3a (150 mg/kg)	156.75 ± 2.05	165.75 ± 2.05	5.25 ± 0.48
3f (100 mg/kg)	180.5 ± 5.57	222.5 ± 8.29	8.25 ± 1.03
3 g (150 mg/kg)	174.25 ± 9.35	183 ± 8.21	4.62 ± 0.75

n = 5; mean ± SEM; −ve = decrease in body mass (all other values showed increase in body mass).

Statistical Analysis

The sum results of the obtained data were expressed as mean ± SEM (standard error of mean). Parametric data were assessed by one-way ANOVA (analysis of variance) followed by Dunnett's test. Graphics and statistical hypothesis testing were done using Graph Pad Prism version 5.0 and IBM SPSS version 19. Values $P < 0.05$ were considered as statistically significant.

Results

Preliminary Screening of In-vivo *Hepato Protective Activity of Test Compounds*

Of the 7 test compounds, 3 chalcones (**3a**, **3f** and **3 g**) showed promising hepatoprotective activity as indicated by significant reduction in liver enzymes (ALT, AST & ALP) and serum bilirubin levels in 4-acetaminophenol induced hepatotoxic rats (Table 1). The rest of the test compounds did not show convincing results and hence were not included in the subsequent testing.

Hepatoprotective and Antioxidant Activities of Selected Chalcone Analogues

Effect on body weight. There was substantial degree of weight loss on PCM administration in animals. However, in animals treated with test compounds and the reference drug showed an increase in the body weight as summarized in Table 2.

Effect on liver enzymes and total bilirubin. The 4-acetaminophenol administration in rats caused significant elevation in levels of liver enzymes resulting in their decreased activity when compared with normal the results of normal control group rats. Serum total bilirubin concentration was also increased in toxic group rats compared to normal control rats. Administration of the selected test **chalcones 3a, 3f** and **3 g** exhibited a statistically significant ($p \leq 0.05$) reduction in levels of liver enzymes toward normal and comparable to their levels in silymarin administered rats. Reduction in levels of liver enzymes indicated the increased activity of these enzymes. Serum total bilirubin concentration was also reduced in chalcone treated rats. The results of liver enzymes and serum total bilirubin in different study group animals are given in Table 1.

Oxidative Stress Markers

Antioxidant enzymes activities including catalase (CAT) and superoxide dismutase (SOD) were measured as markers of oxidative stress while malondialdehyde (MDA) was determined as lipid peroxidation marker. Significant variation in oxidative stress markers have been observed in different study group animals. Results showed that selected chalcone analogues **3a, 3f** and **3 g** antagonized the effect of 4-acetaminophenol, thus increased the antioxidant enzymes catalase and superoxide dismutase whereas reduced MDA indicated the decreased lipid peroxidation. Catalase and SOD was decreased in 4-acetaminophenol induced rats while MDA was found increased in toxic group rats compared to control groups and chalcone administered group rats (Figure 2).

In vitro antioxidant activities of the test synthetic chalcone analogues were determined by DPPH free radical scavenging method using ascorbic acid as standard. The tested synthetic chalcone analogues exhibited significant ($p \leq 0.05$) antioxidant activities (Figure 3).

Histological Examination of Liver

The histological examination of the liver tissues collected from study rats revealed extensive necrosis of hepatocytes and fatty liver in 4-acetaminophenol induced hepatotoxic rats in the toxic group rats while improvement in the histoarchitecture of liver tissues were found in chalcone treated rats in test groups animals. The microscopic picture of H & E stained sections of the liver tissues at 400x magnification using 40x objective lens. Figure 4 showing the micrograph of H & E stained liver tissue of rats from different groups. (**a**) Toxic group rats with 4-acetaminophenol induced toxicity showing necrosis in the liver tissue, (**b)** Normal control group rats showing normal histoarchitecture of liver tissue, (**c)** Rats treated with silymarin showing improved histomorphology of liver tissue indicating hepatoprotective protective potential of silymarin, (**d-f)** Rats treated with test compounds **3f, 3 g** and **3a**

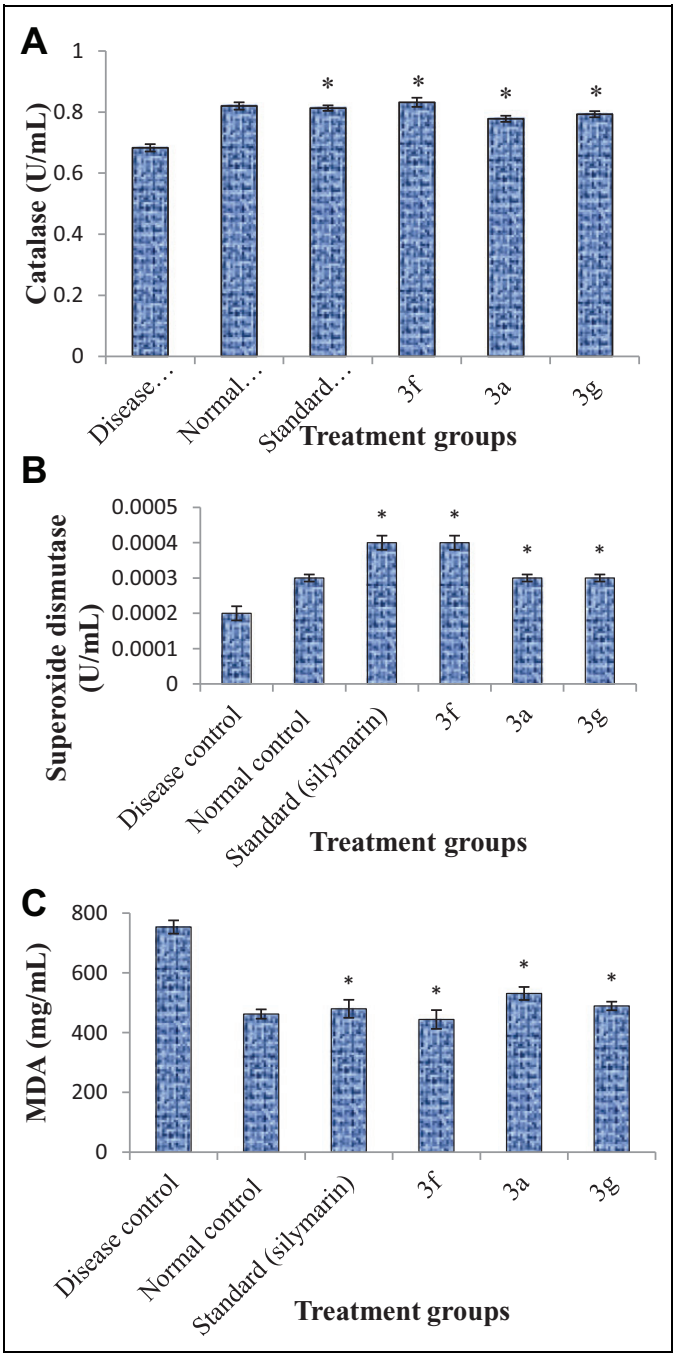

Figure 2. Effects of test chalcones (**3a**, **3f** and **3 g**) on serum antioxidant parameters: (A) Catalase (CAT), (B) Superoxide dismutase (SOD) and (C) Malondialdehyde (MDA) in 4-acetaminophenol-induced hepatotoxic rats. Values are expressed as means ± SEM, n = 5; One way ANOVA followed by Dunnett's multiple comparison test was used; * = $P \leq 0.05$ (significant results).

showing improvement in architecture of liver tissue from rats in test groups **3f, 3 g** and **3a**, respectively.

Discussion

Chalcones are secondary metabolite of medicinal plants with similar chemical structure as that of curcumine, a well known

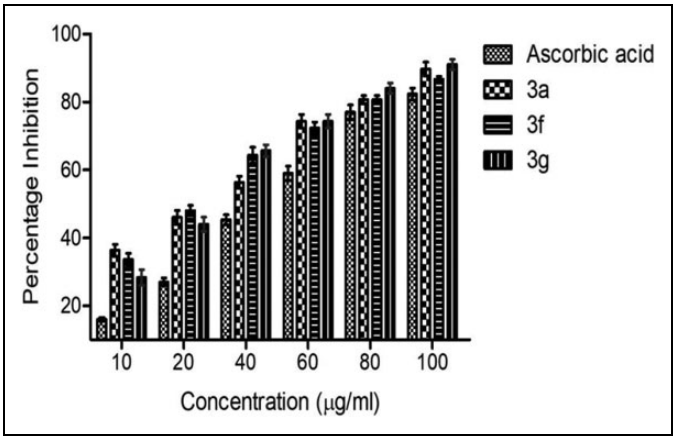

Figure 3. Showing mean % inhibition of ascorbic acid and test compounds (**3a**, **3f** and **3 g**) by using DPPH method at various concentrations. X-axis shows different concentration (µg/mL) and y-axis shows % inhibition. Values are expressed as Means ± SEM; n = 5.

antioxidant. The characteristics of chalcones are related to antioxidant capacity and capturing of the metallic ions.[23] Natural chalcones bearing 3,4-dihydroxyl groups, such as butein, sappanchalcone and okanin are particularly effective antioxidants.[24] The present study was conducted to evaluate the hepatoprotective and antioxidant activities of newly synthesized chalcone analogues in 4-acetaminophenol-induced hepatotoxicity in experimental rats.

Preliminary screening of 7 newly synthesized chalcones revealed that 3 chalcones were potent hepatoprotective in 4-acetaminophenol-induced hepatic damage in experimental rats and thus selected for further testing. Body weight is highly sensitive variable in toxicity studies and taken into consideration when noxious effects of several drugs are going to be determined.[25] The treatment of animals with test chalcone analogues showed a substantial increase in body weight which reflected the improvement in general health conditions of the animals. Literature showed that liver enzymes and total bilirubin levels have been determined to evaluate hepatic damage.[26] The 3 chalcone analogues (**3a**, **3f** and **3 g**), selected in initial screening, showed considerable hepatoprotective activity even at higher doses (100, 150, 150 mg/kg) as revealed by significant reduction in the levels of liver enzymes ALT, AST & ALP and serum total bilirubin concentration. It is pertinent to mention that the hepatoprotective effects were dose dependent as evident from more reduction in liver enzymes and bilirubin than observed in preliminary screening. These hepatoprotective effects were comparable to the reference drug i.e. silymarin as well as with previously reported hepatoprotective activities of existing hepatoprotective agents.[9] However, the elevated liver enzymes as seen in disease control were due to 4-acetaminophenol. Mechanistically, liver damage involves free ROS formation which is associated with the glutathione and tocopherol depletion thereby minimizing liver's oxidative capacity and raised NAPQI concentration.[8]

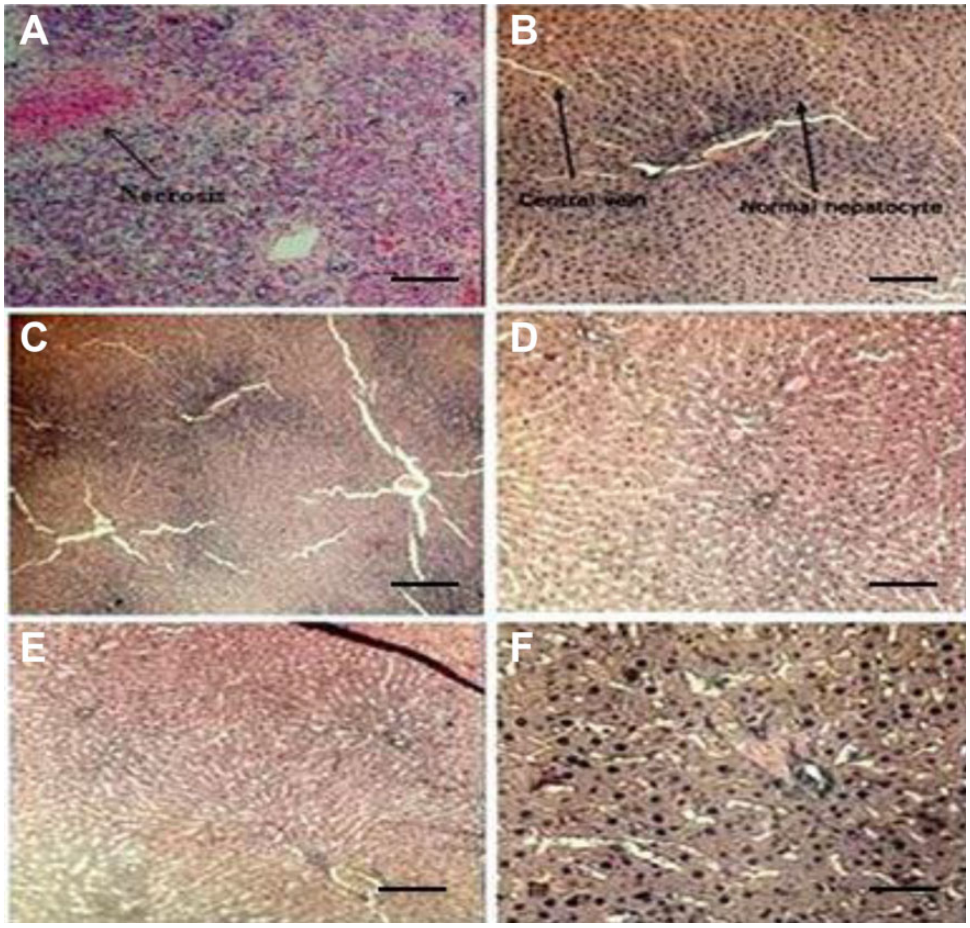

Figure 4. H & E stained sections of liver tissue of rats from different study groups. Scale bars of 5 μ thin sections. A, Toxic group rats with 4-acetaminophenol induced toxicity showing necrosis in the liver tissue. B, Normal control group rats showing normal histoarchitecture of liver tissue. C, Rats treated with silymarin showing improved histomorphology of liver tissue indicating hepatoprotective protective potential of silymarin. D-F, Rats treated with test compounds **3f, 3 g & 3a** showing improvement in architecture of liver tissue from rats in test groups **3f**, **3 g** and **3a**, respectively.

In order to prove the concept that the hepatoprotective effects of test chalcones were due to their antioxidant properties, activities of MDA, Catalase and SOD were determined using standard methods. According to the literature, serum MDA levels were increased whereas CAT and SOD levels were decreased in hepatic damage.[27] In the present study, significant reduction in serum MDA and elevation in CAT and SOD levels followed by administration of the test compounds reflected their antioxidant activity that was comparable with standard hepatoprotective drug, Silymarin. *In vitro* antioxidant potential of test chalcones determined by DPPH free radical scavenging method, showed significant ($p \leq 0.05$) antioxidant activities. The antioxidant activities of the test compounds was also consistent with the literature available in context of antioxidant effects of herbal agents.[28]

The above discussed findings of the biochemical parameters were also supported by the histological examination of liver tissue collected from study groups animals. The 4-acetaminophenol treated rats showed necrosis of hepatocytes, manifested by disappearance of nuclei and aggregation of inflammatory cells as compared to control group, that might due to the formation of free radicals and oxidative stress induced by 4-acetaminophenol and this was quite consistent with the available literature.[5] In addition, the treatment of animals with test chalcones (**3a**, **3f** and **3 g**) caused restoration of normal hepatocyte morphology which further confirmed the hepatoprotective effects that was comparable with the group of rats treated with reference drug, silymarin. The chalcone analogue **3f** showed the hepatoprotective activity at lower dose (100 mg/kg) as compared to other 2 analogues hence can be considered as most potent among 3 test chalcones. Our results were in agreement with previous reports on natural hepatoprotective agents.[29] Therefore, hepatoprotective mechanism of chalcones might be due to bioactive antioxidant compounds that enhances the antioxidant defense system by free radical scavenging activity. Furthermore, histomorphology of liver tissue of test compound

treated groups animals were found normal as compared to 4-acetaminophenol-induced disease control rats. Based upon the findings of the present investigation, it is quite evident that 3 compounds are pharmacologically effective for the treatment of liver disorders when compared with silymarin.

Conclusion

It can be concluded from the above discussion that all the 3 chalcones; **3a**, **3f** and **3 g** possessed significant hepatoprotective and antioxidant activities against 4-acetaminophenol-induced hepatic injury in rats. It was also confirmed by possible reduction in inflammation and improvement in histological changes in liver tissues. However, there are some limitations in this study like the acute toxicity studies were not conducted due to limited quantity of the test synthetic compounds. Detailed studies are required to establish the safety of test compounds and to elucidate the exact mechanism of action responsible for hepatoprotective effects.

Acknowledgments

The authors are very thankful to College of Pharmacy, University of Sargodha, Sargodha, Pakistan, for financial assistance and providing the platform support.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Received financial support from College of Pharmacy, University of Sargodha, Sargodha, Pakistan.

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