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Research Article

Hepatoprotective Effect of Combination of *Curcuma domestica* Val and *Phyllanthus niruri* Linn Against Paracetamol–Induced Liver Damage in Wistar Rats

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ABSTRACT

The present study was carried out to evaluate the hepatoprotective effect of the combination of *Curcuma domestica* Val and *Phyllanthus niruri* Linn extract against paracetamol-induced liver injury in Wistar rats. The different groups of animals were administered with vehicle (Na CMC 0.5%), sylimarin (100 mg/kg BW), *C. domestica* (100 mg/kg BW), *P. niruri* (200 mg/kg BW), combination of *C. domestica* and *P. niruri* (75:50; 50:100; and 25:150 in mg/kg BW) for 7 consecutive days. Thirthy minutes after last treatment all groups were terated with paracetamol (2.5 g/kg BW). The effect of sylimarin, *C.domestica*, *P. niruri*, and its combinations on serum transaminase (ALT, AST), alkaline phosphates (ALP), bilirubin, and total protein were measured in paracetamol induced hepatotoxicity in rats. Further, the effects of the extracts or combinations of extracts on liver gluthatione content, lipid peroxidation (LPO), and catalase (CAT) were estimated. Sylimarin, *C.domestica*, *P. niruri*, and its combinations produced significant (P<0.05, P<0.01, P<0.001) hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin, and lipid peroxidation and significantly (P<0.05, P<0.01, P<0.001) increased the levels of total protein, CAT, and GSH. The optimum combination dose of *C. domestica* and *P. niruri* was 75:50 in mg/kg BW compare to the extracs alone. It was suggested that the combination of *C. domestica* and *P. niruri* showed significant hepatoprotective effect in paracetamol-induced liver injury in Wistar rats.

Keywords: Hepatoprotective, *C. domestica*, *P. niruri*, paracetamol, Wistar rats

INTRODUCTION

Liver has a pivotal role in regulation of physiologial processes. It is involved in several vital functions such as metabolism, secretion and storage. Liver plays an important role in drug elimination and detoxification, but in turn, it can be subjected to damage by xenobiotics Liver diseases are mainly caused by toxic hemicals (antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorder. Almost of all the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative liver damages in liver[1,2]. Currently the no more available drugs for treatment of liver disorders, which make a consideration to develop new drugs with less side effect.

Paracetamol is a drug with analgetic effects and antipyretic is safe used at doses below 4,0 g/hari[3]. Several evidences reported that paracetamol in high dose (15 g/day) can cause liver damage[3,4]. Paracetamol is metabolized by the enzyme process undergoes cytochrome P450 into reactive metabolites that are known as *N*-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI is capable to interact with covalent macromolecule hearts on the cysteine and lipid oxidation and led to cause damage to the liver[5]. Paracetamol used in toxic dose can decrease level of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH). Liver damaged caused by parasetamol can lead increase level serum of glutamate pyruvate transaminase (SGPT), glutamate oxaloacetat transaminase (SGOT), serum alkaline phosphatase (ALP), billirubin, total protein, dan gama glutamintransferase (γGT)[6,7,8].

Several lines of evidence reported that turmeric (*C. domestica* Val) has hepatoprotective activity for several hepatotoxin like paracetamol, galactosamine, CCl_4, thioacetamide[9,10]. Previous study reported that turmeric can prevent fatty liver and necrosis[11]. Curcumin, the active constituent in *C. domestica* exerted hepatoprotective effects of liver injury in various animal models against toxic effects of agent such as carbon tetrachloride[12,13], endotoxin[14] and thioacetamide[15]. In other hand, *P. niruri* Linn also has hepatoprotective activity for several hepatotoxin like parasetamol and CCl_4[16,17,18]. *P. niruri* also can decrease oxidative stress and liver damage caused by alcohol and polyunsaturated fatty acid[19].

To our knowledge, no study has so far reported the hepatoprotective activity using combination of *C. domestica* and *P. niruri* against the hepatotoxin

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paracetamol. It interesting to investigate the hepatoprotective effect of combination of *C. domestica* and *P. niruri* on biochemical parameters (ALT, AST, ALP, billirubin and total protein) on rats–induced paracetamol. The oxidative stress parameters were also obeserved include parameters of lipid peroxidation (LPO), glutathione (GSH), and catalase (CAT). The combination of *C. domestica* and *P. niruri* may offered promising as hepatoprotective agent.

MATERIALS AND METHODS

Materials

C. domestica Val and *P. niruri* Linn ethanol extracts were obtained from Gama Herbal Industries Co. These plants were authenticated at Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. The voucher specimen was stored in a herbarium of the department. The dried leaves were powdered and then stored in an airtight container for further use. Paracetamol, glutathione, *thiobarbituric acid* (TBA) and Ellman's reagent (5,5'-dithio *bis*-2-nitrobenzoic acid), H_2O_2, were purchased from E. Merck, Darmstadt, Germany. Alanine transaminase (AST), Aspartat transaminase (AST), alkaline phosphatase (ALP), bilirubin, and total protein were analyzed using commercial reagent kits (DiaSys Diagnostic, Holzheim, Germany). All other reagents were of analytical grade. Histophatology liver analyze hematoxylin, eosin, paraffin, formalin were puchased from Sigma (St. Louis, MO, USA).

Plant Preparation and Ethanolic Extraction

Fresh plants were harvested and washed thoroughly with running tap water and then with distilled water. The rhizome of *C domestica* or *P niruri* herb were air dried and then oven dried under reduced temperature. The fully dried plants were powdered and weighed before maceration. The powder of *C domestica* or *P niruri* was macerated with ethanol 96% and 70%, respectively. The extracts were filtered before evaporated to dry under reduced pressure at low temperature with a rotary evaporator (Heidolph Instruments GmbH & Co, Schwabach, Germany). The ethanolic extract was further lyophilized by a freeze dryer VirTis BTK (SP Scientific, Gardiner, NY, USA).

Animals

Healthy Wistar rats were obtained from the Animal Experimental Unit, Animal Research Centre, Universitas Gadjah Mada. Animals were maintained in the institutional animal facilities. Animals were housed and maintained under the standard conditions of 12-h light/dark cycle, 25 ± 2°C and 60–70% humidity and allowed free access to standard pellet diet and reverse osmosis water ad libitum. Rats were acclimatized to laboratory condition for 1 week before commencement of experiment. All the rats were acclimatized quarantined for one week prior to experimentation. The experimental protocol was conducted in accordance with the Guideline for Care and Use of Animals Laboratory and approved by the Institutional Animal Ethics Committee (IAEC) of Gadjah Mada University.

Hepatoprotective study

Healthy forty-five Wistar albino rats were divided into 8 groups each containing 5 animals. Group I (control) administered with vehicle (Na CMC 0.5 %, p.o.). Group II administered vehicle (Na CMC 0.5 %, p.o.) for 7 days and 30 minutes after last sample adminstration rats were received paracetamol, 2.5 g/kg BW, p.o. Group III administered sylimarin (100 mg/kg BW, p.o.) for 7 days and 30 minutes after last sample administration rats were received paracetamol, 2.5 g/kg BW, p.o. Group IV-VIII administered *C.domestica* only (100 mg/kg BW), *P.niruri* only (200 mg/kg BW), as well as a combination of *C.domestica* and *P.niruri* (75:50; 50:100; and 25:150 mg/kg BW, p.o.), respectively for 7 days and 30 minutes after last sample administration rats were received paracetamol, 2.5 g/kg BW, p.o. After 48 hours of paracetamol administration, the blood was collected through retro-orbital plexus for determination of ALT, AST, ALP, bilirubin and total protein. Finally, animals were sacrificed and liver quickly removed and excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. Some part were fixed on formaline 10% for histological observation. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer. A part of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for estimation of glutathione by the method of Ellman (1959)[20]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of catalase (CAT) activities was measured by the method of Aebi (1974)[21].

Estimation of GSH

The procedure to estimate the reduced glutathione (GSH) level followed to the method as described by Ellman (1959)[20]. The homogenate (in 0.1 M phosphate buffer, pH 7.4) was added with equal volume of 20% trichloroacetic acid (TCA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200 µL) was then transferred to a new set of test tubes and added 1.8 mL of the Ellman's reagent (5, 5'-dithio *bis*-2-nitrobenzoic acid) (0.1 mM) was prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes make upto the volume of 2 mL. After completion of the total reaction, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH. The glutathione level in liver was calculated as micromol/g liver.

Estimation of CAT

Catalase (CAT) activity was measured by the method of Aebi (1974)[21]. Supernatant (0.1 mL) was added to cuvette containing 1.9 mL of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 mL of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as specific activity as unit/mg protein or total activity as unit/mg liver.

Lipid peroxidation

Table 1: Effect of *combination of C.domestica and P. niruri* on serum enzymes ALT, AST and ALP on paracetamol–induced liver damage in rats[a].

Group	ALT (U/l)	AST (U/L)	ALP (U/L)
Normal	64.0 ± 3.51	168.8 ± 6.17	85.8 ± 10.02
Control (Paracetamol 2.5 g/kgBW)	307.0 ± 33.79$^{###}$	385.4 ± 38.15$^{###}$	184.2 ± 6.12$^{###}$
Sylimarin (100 mg/kg BW)+ Paracetamol	83.0 ± 8.37***	183.8 ± 25.05***	137.6 ± 6.73**
C.domestica (100 mg/kg BW) + Paracetamol	115.6 ± 3.51***	196.8 ± 26.70**	125.6 ± 4.15***
P.niruri (200 mg/kg BW)+ Paracetamol	121.6 ± 23.13***	206.0 ± 31.96**	142.6 ± 11.04*
C.domestica and P. niruri (75:50 mg/kg BW) + Paracetamol	96.2 ± 13.96***	209.8 ± 36.26**	101.0 ± 7.79***§
C.domestica and P. niruri (50:100 mg/kg BW) + Paracetamol	123.6 ± 20.04***	192.4 ± 24.38**	109.2 ± 10.49***
C.domestica and P. niruri (25:150 mg/kg BW) + Paracetamol	153.8 ± 21.39***	244.4 ± 29.97*	97.4 ± 4.57***§

[a]Data represent mean ± standard error of the mean of 5 rats.
**P*<0.05, ***P*<0.01, ****P<0.001, significantly different with control paracetamol group; $^{###}$*P*<0.001, significantly different with normal group; §*P*<0.05, significantly different with *P. niruri* alone group.

A part of liver tissues were then homogenized in 0.1 M buffer pH 7.4) with a Teflon-glass homogenizer. Lipid peroxidation was determined by measuring the amounts of malondialdehyde (MDA) produced primarily, according to the method of Ohkawa et al. (1979)[22]. The 0.2 mL of tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL 20% acetic acid and 1.5 mL 8% TBA were added. The volume of the mixture was made upto 4 mL with distilled water and then heated at 95°C on a water bath for 60 min. After incubation the tubes were cooled to room temperature and final volume was made to 5 mL in each tube. Five mL of butanol:pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its OD read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of $1.56 \times 10^{5}\ M^{-1}\ cm^{-1}$.

Histopathological Evaluation.

Tissues were dehydrated in ascending grades of ethanol (70% to 100%) and cleared in xylene with the use of TP1020 automated tissue processor (Leica, Germany) after fixing in 10% formalin for overnight. Then, the tissues were embedded in paraffin wax blocks on the EG 1140 H embedding station (Leica, Germany), section to 4 µm thick using HM 340E Electronic Rotary Microtome (Thermo Scientific, USA) and mounted on glass slides. After that, deparaffination was carried out by immersing the slides in Xylol for 3 minutes and then in 100% ethanol for 1 minute, 96% ethanol for 1 minute, 80% ethanol for 1 minute, and 60% ethanol for 1 minute. Lastly, the slides were stained with haematoxylin (Sigma, St. Louis, MO, USA) and eosin (Sigma, St. Louis, MO, USA) and then mounted under coverslips using DPX mounting medium (BDH Laboratory, England). Histological changes in the tissue sections were examined and captured under the BX51 light microscope (Olympus, Japan).

Statistical analysis

The data are presented as the mean ± SEM. The statistical significance of differences between the groups were assessed with a one-way ANOVA, followed by Bonferroni post-hoc test analysis using GrapPad InStat 3 (GraphPad Software, Inc., USA). **P*<0.05, ***P*<0.01, ****P*<0.001 considered statistically significant.

RESULTS

Effect of combination of C.domestica and P. niruri on serum ALT, AST, ALP, bilirubin, and total protein

The effect of combination of *C.domestica* and *P. niruri* on ALT, AST, ALP, bilirubin, and total protein is showed in Table 1. The result demonstrated that ALT, AST, ALP, bilirubin, and total protein were found to be significantly increased in rats treated with paracetamol when compared with the normal group (*P*<0.001). In other hand, the administration of paracetamol was significantly reduced total protein level in serum (*P*<0.001). The administration of for *C.domestica* or *P. niruri* alone for 7 days was significant decreased the activity of serum ALT, AST, ALP and bilirubin in paracetamol-induced liver damage in rats compared to that of hepatotoxic group (paracetamol treatment) (*P*<0.05, *P*<0.01, *P*<0.001). Further, the combination of *C.domestica* and *P. niruri* were also significantly decreased the activity of serum ALT, AST, ALP and bilirubin in paracetamol-induced liver damage in rats compared to that of hepatotoxic group (paracetamol treatment) (*P*<0.05, *P*<0.01, *P*<0.001). The optimum hepatoprotective effect of combination of of *C.domestica* and *P. niruri* was achived on combintaion *C.domestica* and *P. niruri* (75:50 mg/kg BW). The administration of *C.domestica* and *P. niruri* alone or combination were significantly recovered to the normal content of total protein. In the combination of of *C.domestica* and *P. niruri*, the increase dose of *P. niruri* was reduce the hepatoprotective effect. In the present study, *C. domestica* have important role than *P. niruri* in hepatoprotective effect.

Effect of combination of C.domestica and P. niruri on lipid peroxidation

Table 2: Effect of *combination of C.domestica and P. niruri* on serum bilirubin and total protein on paracetamol−induced liver damage in rats[a].

Group	Bilirubin (mg/dL)	Total Protein (mg/dL)
Normal	0.10 ± 0.02	8.00 ± 0.17
Control (Paracetamol 2.5 g/kgBW)	0.24 ± 0.04$^{\#\#}$	5.44 ± 0.34$^{\#\#\#}$
Sylimarin (100 mg/kg BW)+ Paracetamol	0.08 ± 0.02***	7.10 ± 0.24*
C.domestica (100 mg/kg BW) + Paracetamol	0.10 ± 0.02**	7.10 ± 0.26*
P.niruri (200 mg/kg BW)+ Paracetamol	0.12 ± 0.01*	7.16 ± 0.43*
C.domestica and P. niruri (75:50 mg/kg BW) + Paracetamol	0.08 ± 0.02***	7.14 ± 0.18*
C.domestica and P. niruri (50:100 mg/kg BW) + Paracetamol	0.08 ± 0.02***	7.22 ± 0.46**
C.domestica and P. niruri (25:150 mg/kg BW) + Paracetamol	0.10 ± 0.02**	6.86 ± 0.19

[a]Data represent mean ± standard error of the mean of 5 rats.
*P<0.05, **P<0.01, ****P<0.001, significantly different with control paracetamol group; $^{\#\#}P$<0.01, $^{\#\#}P$<0.01, significantly different with normal group.

The localization of radical formation resulting in lipid peroxidation, measured as MDA in rat liver homogenate is showed in Table 2. The MDA content in the liver homogenate was significantly increased in paracetamol-treated group compared to normal group (P<0.001). MDA level of *C.domestica* or *P.niruri* alone, were significantly reduced compared to paracetamol-treated group (P<0.01, P<0.001). At the same time, the effect of combination of *C.domestica* and *P. niruri* also decreased on MDA levels in paracetamol induced hepatotoxicity in rats (P<0.01, P<0.001). The optimum combination dose of *C. domestica* and *P. niruri* was 75:50 in mg/kg BW compare to the extracs alone.

Effect of combination of C.domestica and P. niruri on GSH content in liver tissues

The result in Table 3 showed the effect of *C. domestica* or *P. niruri* and its combinations on glutathione content in the liver. The content of GSH level in liver in hepatotoxic group (paracetamol-treated group) was significantly depleted compare to normal group (P<0.001). The administration of *C. domestica* or *P. niruri* and its combinations were significantly restore the GSH content of liver (P<0.01) against paracetamol−induced GSH depletion. There are no significantly different between extract alone or combination on effect on GSH depletion. Therefore, *C. domestica* or *P. niruri* and its combinations capable to protect the depletion of GSH content against paracetamol−induced liver damaged.

Effect of combination of C.domestica and P. niruri on Catalase activity in liver tissues

The result demonstrated that CAT activity in liver of paracetamol-treated group was significantly lower than normal group (Table 3) (P<0.05). The administration of *C. domestica* or *P. niruri* and its combinations significantly increased CAT activity compared to paracetamol-treated group (P<0.05). Interestingly, the administration *P. niruri* alone (200 mg/kg BW, sylimarin (100 mg/kg BW), and combination *C.domestica* and *P. niruri* (75:50 mg/kg BW), not only completely restored the CAT activity to the normal level, but also stimulated the CAT activity than normal group. The results suggest that *P. niruri* alone (200 mg/kg BW, sylimarin (100 mg/kg BW), and combination *C.domestica* and *P. niruri* (75:50 mg/kg BW), capable to protect the reduction of CAT against paracetamol−induced liver damaged in rats.

Effect of combination of C.domestica and P. niruri on histopathology of liver

The results of histopathological examination of *C. domestica* or *P. niruri* and its combinations on paracetamol-treated rats are shown in Figure 1. In control rats, liver sections showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus and central vein. In paracetamol-treated rats, liver there were multifocal necrosis in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils. In *C. domestica* or *P. niruri* and its combinations-treated rats, liver sections showed residual hepatocellular necrosis with cords of regenerating hepatocytes.

DISCUSSION

Paracetamol, a widely used non-prescription analgesic-antipyretic drug, is safe when used within therapeutic doses. When taken in large doses, it may cause hepatotoxicity, leading to fulminant hepatic and renal tubular necrosis, which is lethal to humans and many species of animals[23,24,25]. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates[26,27]. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450[28,29], to a highly reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI). Induction of cytochrome P450 or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity. The covalent binding of NAPQI, a highly reactive metabolite product of paracetamol, to sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier[30,31].

The assessment enzymes level such as ALT and AST is

Table 3. Effect of *combination of C.domestica and P. niruri* on gluthatione (GSH) content, lipid peroxidation (LPO), and catalase on paracetamol–induced liver damage in rats[a].

Group	GSH (μM/g liver)	LPO (nM MDA/ mg protein)	Catalase (U/mg protein)
Normal	35.22 ± 1.05	2.50 ± 0.29	3.79 ± 1.12
Control (Paracetamol 2.5 g/kgBW)	7.95 ± 1.13$^{\#\#\#}$	3.74 ± 0.10$^{\#\#\#}$	0.66 ± 0.31$^{\#}$
Sylimarin (100 mg/kg BW)+ Paracetamol	35.14 ± 5.46***	2.71 ± 0.12*	13.70 ± 4.46*
C.domestica (100 mg/kg BW) + Paracetamol	26.88 ± 2.72**	2.13 ± 0.23***	3.33 ± 0.54*
P.niruri (200 mg/kg BW)+ Paracetamol	29.68 ± 5.99**	2.61 ± 0.19**	10.41 ± 2.77*
C.domestica and P. niruri (75:50 mg/kg BW) + Paracetamol	28.93 ± 4.58**	1.98 ± 0.20***	12.54 ± 3.19*§
C.domestica and P. niruri (50:100 mg/kg BW) + Paracetamol	29.95 ± 4.52**	2.55 ± 0.14**	5.31 ± 1.78*
C.domestica and P. niruri (25:150 mg/kg BW) + Paracetamol	23.74 ± 1.38**	2.43 ± 0.08***	2.45 ± 0.56*

[a]Data represent mean ± standard error of the mean of 5 rats.
*P<0.05, **P<0.01, ****P<0.001, significantly different with control paracetamol group; $^{\#}P$<0.05, $^{\#\#\#}$P<0.001, significantly different with normal group; $^{\S}P$<0.05, significantly different with *C. domestica* alone group.

largely common used to assess liver damage induced by paracetamol. Necrosis or membrane damage releases the enzyme into circulation. High levels of AST indicates liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury[31]. The elevated levels of serum enzymes transaminase are indicative of cellular leakage and loss of functional integrity of cell membrane in liver[32]. Serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure[33].

This present study evaluated the hepatoprotective effects of *C.domestica* or *P.niruri* and its combinations in paracetamol induced liver damage in rats. Acute administration of paracetamol produced a marked elevation of the serum levels of ALT, AST, ALP, bilirubin and total proteins in treated animals when compared with that of normal group. Treatment with *C.domestica* or *P.niruri* and its combinations decreased the serum levels of ALT, AST towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. The above repairment can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. The effect on alkaline phosphatase (ALP) and bilirubin levels are points towards an early improvement in the secretary mechanism of the hepatic cell.

Oxidative stress is considered to be associated with many diseases, including cell damage, but diet plays an important role in human health and in the prevention of certain diseases. Lipid peroxidation has been postulated as being a destructive process in liver injury caused by paracetamol administration[33]. The co-incidence of antioxidant activity and protective effect on liver tissues after paracetamol administration suggest that both free radical generation and lipid peroxidation may be involved in this type of drug injury process[34]. In the present study, elevations in the levels of end products of lipid peroxidation in liver of rat treated with paracetamol were observed. The increase in MDA level in liver suggests provoked lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with *C.domestica* or *P.niruri* and its combinations significantly reversed these elevations.

Glutathione (GSH) is an intracellular reductant and plays major role in catalysis, metabolism and transport. GSH plays a protective role in tissue by detoxification of xenobiotics and essential to maintain structural and functions integrity of the cell. It protects cells against free radicals, peroxides and other toxic compounds. GSH is a naturally occurring substance that is abundant in many living cells. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury[35]. In the present study the decreased level of GSH has been associated with a provoked lipid peroxidation in paracetamol treated rats. Administration of *C.domestica* or *P.niruri* and its combinations significantly increased the level of glutathione in liver, which in turn helps in maintaining the liver tissue damage.The results are consistent with the previous studies that some hepatoprotective compounds has a capability of enhancing GSH level to preserve GSH level[36,37].

Catalase (CAT) is a one of the key component of the antioxidant defense system, widely distributed in tissue and the highest activity is found in the red cells and in liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radical. Therefore, the reduction of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. The accumulation of some free radical may results alterations in the biological activity of cellular

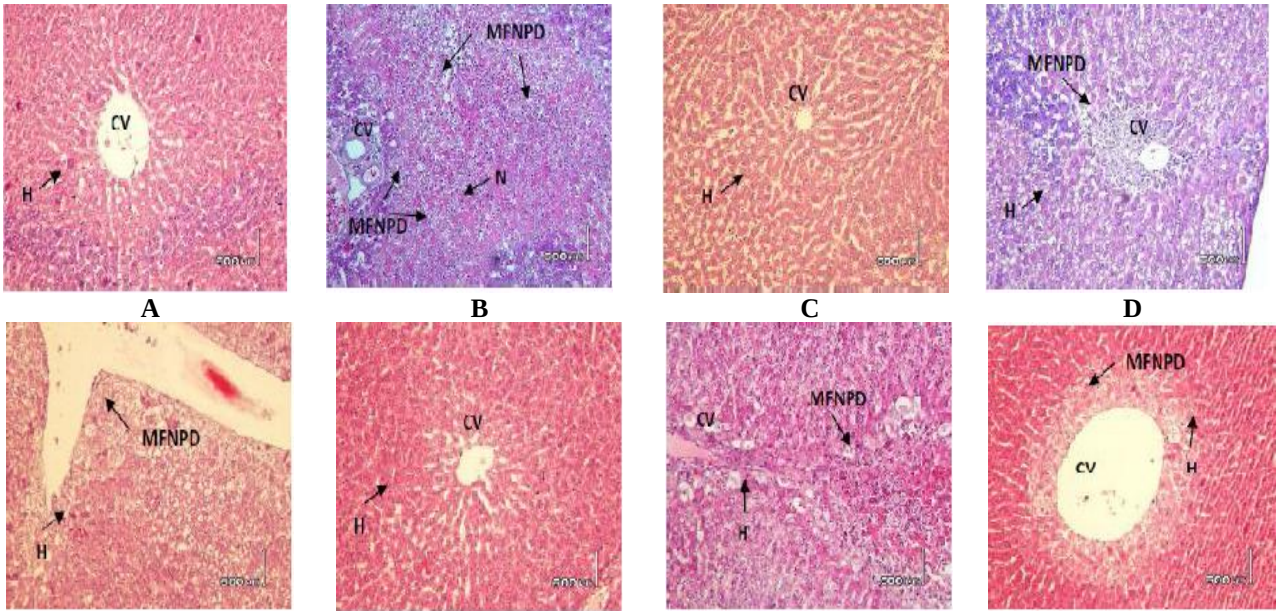

Figure 1: Effect of ***combination of C.domestica and P. niruri*** on histopatological examination on paracetamol induced liver damage in rats. Normal group (A); paracetamol-treated group (B); sylimarin (C); *C.domestica* (D); *P. niruri* (E); combination of *C.domestica* and *P.niruri* (75:50 mg/kg BW) (F); combination of *C.domestica* and *P.niruri* (50:100 mg/kg BW) (G); combination of *C.domestica* and *P.niruri* (25:150 mg/kg BW) (H). CV is central vena; H is hepatocyte; MFNPD is multifocal necrosis near the central vena; N is neutrophile.

macromolecules[38]. Administration of *C.domestica* or *P.niruri* and its combinations increases the activities of catalase to prevent the accumulation of free radicals and protects the paracetamol-induced liver damage in rats.

Curcumin, the most common antioxidant constituent of *Curcuma longa* or *C. domestica* rhizome extract, was reported to enhance apoptosis of damaged hepatocytes which might be the protective mechanism whereby curcumin down-regulated inflammatory effects and fibrogenesis of the liver. The ethanolic extract of *Curcuma Longa* rhizomes showed a significant hepatoprotective effect when orally administrated in doses of 250 mg/kg and 500 mg/kg, and the protective effect was dose dependent. The main constituents of *Curcuma long*a rhizome ethanolic extract are the flavonoid curcumin and various volatile oils, including tumerone, atlantone, and zingiberene[10]. The hepatoprotective effects of turmeric and curcumin might be due to direct antioxidant and free radical scavenging mechanisms, as well as the ability to indirectly augment glutathione levels, thereby aiding in hepatic detoxification. Previous study also reported that curcumin administration decreased MMP-8 expression in liver of paracetamol administered rats. Gene expression measurements revealed that paracetamol decreased the expression of antioxidant genes and increased the expression of interleukin-1β (IL-1β), IL-8, tumor necrosis factor-α (TNF-α) and acute phase proteins. Curcumin administration ameliorated paracetamol-induced alterations in genes expression of antioxidant and inflammatory cytokines[39].

The mechanism of the hepatoprotective effect of *P. niruri* is proposed to be by normalizing ROSs. Additionally, *P. niruri* treatment regulated the expression of transforming growth factor (TGFβ), collagen α1 (Collα1), matrix metalloproteinase-2 (MMP2) and tissue inhibitor of matrix metalloproteinase-1 (TIMP1) genes. In the active fraction of *P. niruri*, the isolated chemical constituents were 4-O-caffeoylquinic acid and quercetin 3-O-rhamnoside[40]. The 4-O-Caffeoylquinic acid, which is classified as a tannin, has been isolated previously from *P. niruri* and proven to possess antioxidant, immunomodulatory, and hepatoprotective effects in several in vivo and in vitro assays[41,42,43]. Quercetin 3-O-rhamnoside has been confirmed in Phyllanthus species and other species of the Euphorbiaceae family[44,45,46] exhibits a wide range of pharmacological benefits including antimicrobial, antiviral, antioxidant, gastroprotective, hepatoprotective, anti-inflammatory and chemopreventive effects[47,48,49,50]. Hence it may be possible that the mechanism of hepatoprotection of *C.domestica* and *P. niruri* is due to its antioxidant effect.

In conclusion, the results of the present study demonstrate that administration *C.domestica* or *P.niruri* and its combinations have a potent hepatoprotective effect upon paracetamol-induced liver damage in rats. The optimum combination dose of *C. domestica* and *P. niruri* was 75:50 in mg/kg BW. These findings would encourage further studies on the pharmacological significance of using these combinations extracts as alternative medicines for prevent hepatotoxin induced liver damage.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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