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PROTECTIVE ROLE OF *PHYLLANTHUS NIRURI* AGAINST NIMESULIDE INDUCED HEPATIC DAMAGE

Mary Chatterjee and Parames C. Sil

Department of Chemistry, Bose Institute, 93/1, Acharya Prafulla Chandra Road, Kolkata-700009 INDIA

ABSTRACT

Present study aimed to evaluate the protective role of the aqueous extract of Phyllanthus niruri (P. niruri) against nimesulide-induced hepatic disorder in mice by determining levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) in serum and also by measuring the hepatic content of the antioxidant enzymes, superoxide dismitase (SOD) and catalase (CAT); the free radical scavenger, reduced glutathione (GSH) and thiobarbituric acid reacting substances (TBARS). Aqueous extract of P. niruri was administered either orally or intraperitoneally in different doses and times as needed for the experiments. Intraperitoneal pretreatment of the extract (100 mg/kg body weight for seven days) reduced nimesulide (750 mg/kg body weight for 3 days) induced increased levels of GOT (37.0 ± 1.8 units/ml in control group vs. 91.8 ± 2.0 units/ml in nimesulide treated group vs. 35.0 ± 1.0 units/ml in extract treated group), GPT (30.0 ± 2.1 units/ml in control group vs. 88.4 ± 2.9 units/ml in nimesulide treated group vs. 34.1 ± 1.8 units/ml in extract treated group), and ALP (7.86 ± 0.47 KA units /ml in control group vs. 23.80 ± 0.60 KA units /ml in nimesulide treated group vs. 7.30 ± 0.40 KA units/ml in extract treated group) to almost normal. In addition, P. niruri restored the nimesulide induced alterations of hepatic SOD (550 ± 20 units/mg total protein in control group vs. 310 ± 13 units/mg total protein in nimesulide treated group vs. 515 ± 10 units/mg total protein in extract treated group), CAT (99.5 ± 2 units/mg total protein in control group vs. 25.0 ± 1.5 units/mg total protein in nimesulide treated group vs. 81.0 ± 0.8 units/mg total protein in extract treated group), GSH (90 ± 3 nmoles/mg total protein in control group vs. 17 ± 4.2 nmoles/mg total protein in nimesulide treated group vs. 81 ± 1 nmoles/mg total protein in extract treated group) and TBARS (measured as MDA, 36.6 ± 3.0 nmoles/g liver tissue in control group vs. 96.3 ± 5.2 nmoles/g liver tissue in nimesulide treated group vs. 41.2 ± 1.7 nmoles/g liver tissue in extract treated group) contents. Dose-dependent studies showed that the herb could protect liver even if the nimesulide-induced injury is severe. Intraperitoneal administration of the extract showed better protective effect than oral administration. Combining all, the data suggest that P. niruri possesses hepatoprotective activity against nimesulide-induced liver toxicity and probably acts via an antioxidant defense mechanism. To the best of our knowledge, this is the first report of the hepatoprotective action of P. niruri against nimesulide induced liver damage.

KEY WORDS

Nimesulide, oxidative stress, hepatotoxicity, Phyllanthus niruri, antioxidant, hepatoprotection

INTRODUCTION

Increasing evidence suggests that liver injury could be induced by the exposure of the body to various pollutants, toxicants, hazardous chemicals and a number of drugs. This major organ, responsible for the metabolism of drugs and toxic chemicals, is also the primary target for many toxic materials causing hepatic disorder (1) mostly by inducing oxidative stress (2-6). Knowledge on the preventive mechanisms against oxidative stress leads scientists to look for biologically relevant compounds, particularly naturally occurring ones, including those used as foods, beverages and herbal plants which can either increase the efficacy of the antioxidants present in our

Address for Correspondence :

Dr. Parames C. Sil
Reader, Department of Chemistry, Bose Institute
93/1, A.P.C. Road, Calcutta-700009
West Bengal, INDIA
E-mail:parames@bosemain.boseinst.ac.in

body or possess the intrinsic antioxidant activity. Herbal plants have been found beneficial to humans as they are the source of various medicinal compounds and some of them possess antioxidant activity (7-12). The herb, *P. niruri* has been and is used widely for the treatment of jaundice (13-15). Recently, Tona et al (16) reported antimalarial activity of *P. niruri in vivo*. In addition, Odetola and Akojenu (17) used this plant in traditional medicine for treatment of diarrhea and other gastrointestinal disorders.

Although a number of reports described the usefulness of this herb against the adverse effects of various drug and toxicants (18-21), no studies have been published to date, describing its beneficiary role against nimesulide-induced hepatotoxicity. The anti-inflammatory drug, nimesulide is a selective cyclooxygenase-2 (COX-2) inhibitor, with only residual activity against cyclooxygenase-1 (COX-1) (22, 23). It is almost exclusively metabolized and cleared by the liver (24). It has been reported that the drug can cause several types of liver damage, ranging from mild abnormal function such as increase in serum amino transferase activity to severe organ injuries such as hepatocellular necrosis or intrahepatic cholestasis (25-28). It is, therefore, of our interest to assess the role of the herb, *P. niruri*, on the nimesulide-induced hepatic disorder.

Present study aimed to investigate the role of *P. niruri* against nimesulide-induced liver toxicity. In this study, the extent of liver damage was assessed by determining the serum levels of various marker enzymes related to hepatic integrity, namely, GOT, GPT, and ALP. Hepatic antioxidant status was evaluated by measuring the hepatic content of SOD, CAT, GSH and TBARS expressed as malondialdehyde equivalents. Dose-dependent studies on both nimesuilde and *P. niruri* were performed to find out the extreme level of toxicity and its prevention induced by the drug and the herb respectively. In addition, oral and intraperitoneal administrations were followed to examine whether administering procedures could make any difference in the hepatoprotective activity of *P. niruri.*

MATERIALS AND METHODS

Chemicals : Nimesulide and Tris-HCl buffer were purchased from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA. GOT, GPT and ALP measurement kits were purchased from Span diagnostics Ltd., India. 5,5'-dithio-bis-nitrobenzoic acid (DTNB), reduced glutathione (GSH), thiobarbituric acid (TBA), nitro blue tetrazolium (NBT), phenazine methosulphate, reduced nicotinamide adenine dinucleotide (NADH), glacial acetic acid, n-butanol were procured from Sisco research laboratory, India.

Plant : *Phyllanthus niruri* is a traditional herbaceous plant of Euphorbiaceae family found in different regions of India and Sri Lanka. Phyllanthus species are also found in other countries of the world including China *(e.g. Phyllanthus urinaria),* Philippines, Cuba, Nigeria and Guam. Leaves, stems and sometimes-whole plant are used for the specified study. Fresh plants were collected from the Bose Institute Experimental Farm and sometimes from local markets.

Preparation of aqueous extract of *P. niruri* : The fresh leaves and stems of the young plants were homogenized in 50 mM sodium phosphate buffer, pH 7.2, at 4^0C and centrifuged the homogenate at 12, 000 x g for 30 minutes to remove unwanted debris. Supernatant was dialyzed against ice-cold water and centrifuged again under the same condition. The supernatant was collected and lyophilized. The freeze-dried material was weighed, dissolved in the same phosphate buffer and used in different experiments.

Test animals : Swiss albino mice (male, body weight 25$\pm$2 g) were used taking into account international principles and local regulations concerning the care and use of laboratory animals. The animals were acclimatized under standard laboratory condition for a period of two weeks before starting any experiment. Animals had free access to standard diet and water *ad libitum*.

Dose and time dependent effect of nimesulide on serum marker enzyme levels : For the determination of dose-dependent effect of nimesulide on liver**,** nimesulide was administered at a dose of 100, 250, 500, 750 and 1000 mg/kg body weight respectively for three days in different groups, each consisted of 8 animals. Based on the result of dose-dependent study, a dose of 750 mg/kg body weight (at this concentration animals showed severe liver damage but survived) was selected for determination of time-dependent nimesulide-induced hepatic damage by treating the animals for one, two, three and five days respectively in different groups.

Determination of the role of *P. niruri* against nimesulide-induced liver damage : Role of *P. niruri* against nimesulide-induced liver damage was determined *in vivo* as described below. The animals were divided into 5 groups, each group having 8 mice. Group I received nimesulide for three days at a dose of 750 mg/kg body weight; groups II and III were pretreated orally and intraperitoneally respectively with the buffer extract of *P. niruri* at a dose of 50 mg/kg body weight twice a day for seven days and then treated with nimesulide for 3 days at a dose of 750 mg/kg body weight. For groups IV

and V, similar procedures were followed like groups II and III except extract was administered at a dose of 100 mg/kg body weight instead of 50 mg/kg body weight. Animals were sacrificed under mild ether anesthesia 24 hours after the last dose of nimesulide administration. Blood and liver of each animal were collected separately. Appropriate controls were used for all the experiments.

Determination of enzyme levels related to hepatic integrity : Blood samples from all the animals of all the study groups were collected separately and allowed to clot at 4°C. Serum was separated by centrifugation. GPT and GOT were assayed spectrophotometrically at 505 nm as described elsewhere (29). ALP was also assayed spectrophotometrically at 510 nm according to the method of Kind and King (30).

Assay of antioxidant enzymes : For the determination of SOD and CAT activities, about 200 mg liver tissue was homogenized in 10 volume of 50 mM potassium phosphate buffer, pH 7.4 containing 1 mM EDTA and a cocktail of different protease inhibitors.

a) *Determination of SOD activity*
SOD activities of all the experimental liver samples were assayed following the method originally developed by Nishikimi et al (31) and then modified by Kakkar et al (32).

b) *Determination of CAT activity*
Hepatic CAT activities of all the experimental samples were analyzed by following the decomposition of H_2O_2 at 240 nm as described by Bonaventura et al. (33). The disappearance of peroxide depending on the catalase activity present in the system was observed at 240 nm for 15 mins. 1 unit of enzyme activity is that which reduces 1 μmole of H_2O_2 per minute (ε=0.0394 $mM^{-1}cm^{-1}$).

Determination of hepatic GSH level : Hepatic GSH level was determined by slight modification of the method of Ellman (34). Briefly, 720 ml of the liver homogenate in 200 mM Tris-HCl buffer, pH 7.2, was diluted to 1440 ml with the same buffer. 160 ml of 5% TCA was added to it and mixed thoroughly. The samples were then centrifuged at 10,000 g for 5 minutes at 4°C. 330 ml of the supernatant was mixed with 660 ml of Ellman's reagent (0.01M DTNB solution). Finally the absorbance was taken at 405 nm.

Estimation of lipid peroxidation level : Extent of lipid peroxidation in the experimental liver samples was determined by using a colorimetric method of the thiobarbituric acid (TBA) reaction as described by Esterbauer and Cheeseman (35). About 1 mg protein contained in liver homogenate in 200 mM Tris-HCl buffer, pH 7.2 was mixed separately with 2.5 ml of TCA-TBA mixture (15% W/V TCA, 0.375% W/V TBA) and kept in a boiling water bath for 30 minutes at 100°C. After cooling, the flocculent precipitate was removed by centrifugation at 1000 xg for 10 minutes. About 99% of thiobarbituric acid reactive substances (TBARS) are malondialdehyde (MDA). The TBARS concentration of the sample was calculated using the extinction coefficient of MDA as 1.56×10^5 $M^{-1}cm^{-1}$.

Measurement of protein concentration : Protein concentrations in the liver homogenates were determined by using a protein estimation kit obtained from Sigma Chemical Company. The estimation was performed according to the method of Bradford (36) using crystalline bovine serum albumin as standard.

Statistical analysis : The results have been expressed as mean ± SD Student's 't' test was employed for all statistical comparisons. Any value $P < 0.05$ was regarded as significant.

RESULTS

Dose-dependent effect of nimesulide on hepatic integrity : Table I shows the result of dose-dependent effect of nimesulide on serum marker enzymes, GPT, GOT and ALP. When administered up to 250 mg/kg body weight, nimesulide did not cause significant change in serum GPT, GOT and ALP level compared to the normal groups (table I). Beyond this dose the enzyme levels increased in a dose dependent manner. Its administration at the dose of 750 mg/kg body weight caused severe enhancement of the serum marker enzyme levels [86.0 ± 2.0 units/ml in nimesulide treated group vs. 29.5 ± 2.3 units/ml in control group for GPT level and 93.0 ± 1.5 units/ml in nimesulide treated mice vs. 37.0 ± 1.8 units/ml in control mice for GOT level and 26.0 ± 0.8 KA units/ml in nimesulide treated group vs. 8.4 ± 0.5 KA units/ml in control group for ALP level] without the loss of any experimental animal. However, at a dose of 1000 mg/kg body weight most of the animals in that particular group died.

Time-dependent effect of nimesulide on hepatic integrity : Results of time-dependent effect of nimesulide on the levels of the serum marker enzymes have been shown in table I. Nimesulide administration at the experimental dose (750 mg/kg body weight) for one day practically did not change the levels of serum GPT and GOT, although ALP level was increased to some extent. However, a linear enhancement was observed for all the enzymes with 2, 3 and 5 days treatment (table I). When nimesulide was administered for 5

days at a dose of 750 mg/kg body weight most of the animals in that particular group seemed to become sick and a few of them died. Results showed that nimesulide at the dose of 750 mg/kg body weight for 3 days induces significant liver damage without any loss of experimental animals. So this dose and time have been used to evaluate the hepatopretective role of *P. niruri* against nimesulide induced liver damage.

Hepatoprotective role of *P. niruri* on nimesulide induced hepatic disorder : Hepatoprotective role of *P. niruri* on nimesulide induced hepatic disorder was evaluated by both the oral and intraperitoneal administration of the extract. This attempt has been adapted to assess whether administration procedures make any difference in the effectiveness of the active ingredient(s) on nimesulide induced liver damage.

Effect of *P. niruri* on serum marker enzymes

On serum GPT and GOT level : Table II shows the effect of the intraperitoneal and oral administration of *P. niruri* on nimesulide-induced GPT and GOT levels. It is evident from the table that the intraperitoneal administration of the aqueous extract at a dose of 100 mg/kg body weight prevented the elevations of GPT (30.0 $\pm$ 2.1 units/ml in control group vs. 88.4 $\pm$ 2.9 units/ml in nimesulide treated group vs. 56.0 $\pm$ 2.8 units/ml in oral administration vs. 34.1 $\pm$ 1.8 units/ml in intraperitoneal administration) and GOT (37.0 $\pm$ 1.8 units/ml in control group vs. 91.8 $\pm$ 2.0 units/ml in nimesulide treated group vs. 61.3 $\pm$ 2.6 units/ml in oral administration vs. 35.0 $\pm$ 1.0 units/ml in intraperitoneal administration) levels more efficiently than those levels by oral administration.

On serum ALP level : Table II shows that pretreatment of mice with *P. niruri* significantly reduced the nimesulide induced elevated level of ALP. It is also evident from the same table that intraperitoneal administration of the extract at a dose of 100 mg/kg body weight is more effective than oral administration in restoring that level (7.86 $\pm$ 0.47 KA units /ml in control group vs. 23.80 $\pm$ 0.60 KA units/ml in nimesulide treated group vs. 13.40 $\pm$ 0.70 KA units/ml in oral administration vs. 7.30 $\pm$ 0.40 KA units/ml in intraperitoneal administration).

Effect of *P. niruri* on antioxidant enzymes

On liver SOD level : Effect of nimesulide alone and effect of pretreatment with *P. niruri* followed by nimesulide administration on SOD activity has been shown in table II. SOD activity in nimesulide treated liver tissue homogenate (310 $\pm$ 13 units/mg total protein) was reduced markedly than the control group (550 $\pm$ 20 units/mg total protein) and pretreatment with *P. niruri* at a dose of 100 mg/kg body weight significantly recovered that SOD depletion. Intraperitoneal administration was found to be more effective in restoring SOD level (515 $\pm$ 10 units/mg total protein) than oral administration (399 $\pm$ 15 units/mg total protein).

On liver CAT level : CAT activity in the nimesulide treated group showed marked reduction compared to normal group (25.0 $\pm$ 1.5 units/mg total protein in nimesulide treated group vs. 99.5 $\pm$ 2 units/mg total protein in control group). As shown in table II, pretreatment with *P. niruri* at a dose of 100 mg/kg body weight, significantly restored the CAT activity and it is higher in the intraperitoneally administered group (81.0 $\pm$ 0.8 units/mg total protein) than the orally treated one (44.0 $\pm$ 1.0 units/mg total protein).

Effect of *P. niruri* on GSH level : GSH levels as measured

Table I : Dose and time dependent hepatotoxic effect of nimesulide in mice

Dose of NIM (mg/kg body weight)	Levels of Marker Enzymes			Time of NIM treatment (days)	Levels of Marker Enzymes		
	GPT (unit/ml)	GOT (unit/ml)	ALP (KA unit/ml)		GPT (unit/ml)	GOT (unit/ml)	ALP (KA unit/ml)
Control	29.5±2.3	37.0±1.8	8.4±0.5	Control	30.1±2.5	33.1±1.9	7.1±0.4
100	32.1±1.3	37.5±1.0	8.2±0.7	1	28.2±1.1	34.0±1.4	8.3±0.3
250	33.9±2.5	40.5±2.0	9.3±0.5	2	47.1±2.5	55.3±3.5	17.9±0.45
500	49.3±3.0*	55.6±2.1*	14.7±1.05*	3	82.1±2.4*	89.0±1.8*	24.8±0.22**
750	86.0±2.0*	93.0±1.5**	26.0±0.8**	5	91.8±2.8*	98.4±1.9**	28.9±0.5**
1000	99.7±2.9*	98.1±2.4**	30.8±1.03*	–	–	–	–

Table I : Dose and time dependent hepato-toxic effect of nimesulide (NIM) on mice. Details have been presented in the materials and method section. For the dose dependent effect of NIM on the levels of marker enzymes, namely, GPT, GOT and ALP, NIM was administered at the doses of 100, 250, 500, 750 and 1000 mg/kg body weight for 3 days. For the time-dependent effect, NIM was administered at a dose of 750 mg/kg body weight for 1, 2, 3 and 5 days. In both the experiments, animals were sacrificed 24 hours after the final dose of NIM administration and the levels of the marker enzymes were determined.

Table II :Effect of the aqueous extract of *P. niruri* on nimesulide induced hepatic damage

Levels of serum marker enzymes, anti-oxidant enzymes, etc	Control	NIM750	Extract of P. niruri			
			PN 50 + NIM		PN 100 + NIM	
			Oral	IP	Oral	IP
GPT (unit/ml)	30.0±2.1	88.4±2.9**	74.6±3.6	59.8±2.3**	56.0±2.8*	34.1±1.8*
GOT (unit/ml)	37.0±1.8	91.8±2.0**	77.1±4.5	60.2±1.6*	61.3±2.6*	35.0±1.0**
ALP (KA unit/ml)	7.86±0.47	23.8±0.6**	19.1±0.9	13.7±0.5*	13.4+0.7*	7.3±0.4**
SOD (unit/mg protein)	550±20	310±13*	348±22	392±9	399+15*	515±10**
CAT (unit/mg protein)	99.5±2.0	25.0±1.5*	28.2±1.7	38.0±1.5*	44.1+1.0*	81.0±0.8**
GSH (nmol/mg protein)	90.0±3.0	17.0±0.2**	29.4±2.9	62.1±1.4**	66.0±3.0*	81.0±1.0**
MDA (nmol/g tissue)	36.6±3.0	96.3±5.2*	81.1±5.6	72.2±2.0*	68.3±5.0*	41.2±1.7**

Table II : Effect of aqueous extract of *P. niruri* on the levels of serum marker enzymes, (GPT, GOT and ALP); antioxidant enzymes, (SOD and CAT), radical scavenger, (GSH) and lipid peroxidation (measured as MDA content) against nimesulide induced hepatic damage. Aqueous extract of *P. niruri* was administered either orally or intraperitoneally 7 days prior to nimesulide treatment. For experimental detail, see the materials and methods. Control: levels in normal mice, NIM 750: levels only in nimesulide treated mice, PN 50 + NIM and PN 100 + NIM: levels in mice liver in which extract of *P. niruri* was given at a dose of 50 and 100 mg/kg body weight respectively prior to nimesulide administration. In all the experiments, animals were sacrificed 24 hours after the final dose of nimesulide administration and the levels of the marker enzymes and other parameters were determined from the sera and tissues respectively. Data represent mean ± SD, n=8; (P*<0.01, P**<0.001).

from the liver tissue homogenates of all the experimental groups have been shown in table II. Nimesulide administration caused massive reduction in liver GSH level (17 ± 4.2 nmoles/mg total protein in nimesulide treated mice vs. 90 ± 3.0 nmoles/mg total protein in normal mice). Intraperitoneal administration with the aqueous extract at a dose of 100 mg/kg body weight for seven days prior to nimesulide treatment significantly elevated that reduction compared to the oral administration (81 ± 1.0 nmoles/mg of total protein in intraperitoneal administration vs. 66 ± 3.0 nmoles/mg of total protein in oral administration).

Effect of *P. niruri* on lipid peroxidation : Effect of *P. niruri* on lipid peroxidation, measured by the hepatic MDA content, has been shown in table II. Those products were tremendously increased in the liver tissue treated with nimesulide compared to that of the control group (96.3 ± 5.2 nmoles/g liver tissue in nimesulide treated mice vs. 36.6 ± 3.0 nmoles/g liver tissue in control mice). Pretreatment with *P. niruri* significantly reduced the nimesulide induced increase in MDA content. It is also evident from the same table that intraperitoneal administration of the extract at a dose of 100 mg/kg body weight is more effective than oral administration (41.2 ± 1.7 nmoles/g liver tissue in intraperitoneal administration vs. 68.3 ± 5.0 nmoles/g liver tissue in oral administration) in restoring the MDA level against nimesulide induced hepatic damage.

DISCUSSION

Present investigation aimed to evaluate the role of *P. niruri* on nimesulide induced hepatic disorder. Using dose- and time-dependent study we found that nimesulide administration at doses of up to 250 mg/kg body weight for 3 days practically did not alter the levels of the serum marker enzymes, GPT, GOT and ALP; beyond this dose, enhancement of the levels of those enzymes (GPT, GOT and ALP) began and at a dose of 750 mg/kg body weight for 3 days, nimesulide caused massive enhancement of the marker enzymes indicating severe liver damage. Beyond this dose (1000 mg/kg body weight or 5 days treatment in our study), most of the animals became sick and some of them died. Since, our goal was to find out whether *P. niruri* could protect the liver from nimesulide-induced severe damage, we decided to conduct experiments by administering nimesulide at a dose of 750 mg/kg body

weight for the specified period of time.

We used two different doses, 50 mg/kg and 100 mg/kg body weight of the aqueous extract of *P. niruri* to determine its role in nimesulide-induced hepatic disorder. Pretreatment with the aqueous extract at a dose of 100 mg/kg body weight prevented nimesulide-induced hepatic damage by significant reduction of the massive elevation of marker enzymes although the dose of 50 mg/kg body weight showed less efficiency. In addition, the herb significantly enhanced nimesulide-induced reduction of the levels of anti-oxidant enzymes SOD and CAT, enhanced the level of non-protein thiol, GSH and reduced the lipid peroxidation suggesting that the herb might preserve the integrity of liver cells. Since the dose of 100 mg/kg body weight of the aqueous extract was a very effective protector against nimesulide-induced hepatic damage, we did not exceed that dose in the present study.

Following a dose-dependent study, we found that nimesulide administration at a dose of 500 mg/kg body weight or beyond significantly elevated the levels of serum GOT, GPT and ALP in the experimental mice compared to those in the normal group. This is likely as several researchers reported that overdoses taken for longer period of time or people having hepatic insufficiency suffer severely from this drug, which results in both immunologic and idiosyncratic reactions that turns the liver from normal physiological to pathophysiological states associated with other problems like hemolytic anemia (28, 37-38). Molecular mechanisms underlying this drug-induced toxicity have not yet been fully elucidated. However, experimental evidence suggests that during metabolism of this type of drug, different reactive metabolites are produced that covalently modify proteins (39), impose oxidative stress (40-41) and causes mitochondrial injury (42).

To counter act the oxidative stress, antioxidant defense mechanism operates in our body to detoxify or scavenge reactive oxygen species (ROS). The antioxidant system comprises different types of functional components including different antioxidant enzymes, together with the substances that are capable of reducing ROS or preventing their formation. Among them, SOD mainly acts by quenching of superoxide (O_2^-), an active oxygen radical (43), produced in different aerobic metabolism. CAT acts by catalyzing the decomposition of H_2O_2 to water and oxygen (44). The non-protein thiol, GSH serves as a scavenger of different free radicals and is one of the major defenses against oxidative stress (45). We observed that nimesulide induced a severe depletion in the hepatic content of SOD, CAT and GSH accompanied by a high level of lipid peroxidation. In other words, nimesulide, in our experiments, induced oxidative stress in liver. Mice administered with *P. niruri* significantly restored the altered levels of those anti-oxidant molecules suggesting that the active ingredient(s) in the herb might possess antioxidant properties and protects liver against nimesulide induced oxidative stress. A very recent study on liver histopathology of nimesulide treated mice and the beneficial role of the extract on the adverse effect of nimesulide showed that nimesulide caused considerable necrosis along the central vein and rendered the sizes of the hepatocytes larger and balloon like compared to those in the normal mice liver. The inflammations in the livers of extract-treated mice on the other hand were less necrotic. Combining all, the data suggest that *P. niruri* possesses protective role against nimesulide induced hepatic damage in mice. Results from our laboratory also showed that oral and intraperitoneal administration of the same amount of aqueous extract in the similar experiments made significant difference in hepatoprotective activity. This is due to the presence of some protein molecules in the herb that might be involved in its hepatoprotective action. The speculation of the involvement of protein components in the extract is due to two reasons; a) when fed orally, the protein molecules might get degraded in the stomach, but intraperitoneal administration keeps them intact and help them to exhibit their function properly and b) results from our preliminary studies showed that heat treatment or enzymatic digestion destroyed the biological activity of the extract.

In conclusion, we would like to mention that the hepatoprotective action of *P. niruri* against nimesulide-induced liver damage might be due to the anti-oxidant properties of the active components present in the aqueous extract of the herb. It is likely that the active ingredient present in the herb would be effective at a very low concentration against nimesulide-induced liver damage and may be an attractive therapeutic tool in future. To have a clear picture about the mechanism of action of *P. niruri* on nimesulide-induced hepatic disorder, further investigation is needed to isolate and characterize the responsible active principle(s) present in it and is currently in progress.

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