

Pharmacological Evaluation of Berberine against Nimesulide Induced Hepatotoxicity

Md. Akram Minhaj¹, Md Shadab Alam²

¹Assistant Professor (Pharmacology), IEC Group of Institution, Greater Noida, India

²Pursuing M.D (Pharmacology), Rajendra Institute of Medical Sciences, Ranchi, India

Abstract: Hepatotoxicity is an injury to the liver that is associated with impaired liver function caused by exposure to a drug or various other agents. Berberine is an isoquinoline alkaloid found in various plants, including *Hydrastis Canadensis* (goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry) and *Berberis aristata* (tree turmeric). The effect of Berberine at 160 mg/kg and 80 mg/kg dose were evaluated by its efficacy to protect against Nimesulide induced hepatotoxicity. After completing 10 days of drug treatment, on day 11, blood was collected through orbital plexus for estimation of various parameters. The measurement of serum SGOT and ALP levels used as a biomarker for diagnosis of liver disease. Nimesulide (100 mg/kg, i.p.) increased serum SGOT, and ALP at the end of 10th day of drug treatment reflecting the liver injury in comparison to control group. The present study found that berberine had both preventive and curative effects on Nimesulide - induced liver damage. Moreover, our findings suggest that dosages may be an important factor for pharmacological effects of berberine. The dosage of Berberine (160 mg/kg, p.o.) has higher efficacy than the doses (80mg/kg, p.o.). Treatment by berberine significantly decreased serum SGOT and ALP. The results demonstrate the hepatoprotective effects of berberine against liver damage induced by nimesulide.

Keywords: Hepatotoxicity, Nimesulide, Berberine, SGOT, ALP

1. Introduction

Hepatotoxicity is an injury to the liver that is associated with impaired liver function caused by multiple factor. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins. There are several specific conditions that all fall within hepatotoxicity. These conditions include: Hepatitis — inflammation of the liver, Hepatic necrosis—death of liver cells, Hepatic steatosis—too much fat in the liver; may be associated with a life-threatening condition called lactic acidosis^{1a}.

Liver dysfunction initiates immunological reactions, including both innate and adaptive immune responses. Hepatocyte stress or damage release signals that stimulate and activate cells of the innate immune system, including Kupffer cells (KC), natural killer (NK) cells, and NKT cells, which contribute liver injury by producing proinflammatory mediators and secreting chemokines. It has also been noted that various inflammatory cytokines, such as tumor necrosis factor (TNF α), interferon (IFN)- γ , and interleukin (IL)-1 β are produced during DILI promotes tissue damage. However, innate immune cells are also the main source of IL-10, IL-6, and certain postganglionic, all of which have been shown to play a hepatoprotective role. Thus, it is the delicate balance of inflammatory and hepatoprotective mediators produced after activation of the innate immune system. In addition to the innate immune responses, clinical features of certain DILI cases strongly suggest that the adaptive immune system is activated and involved in the pathogenesis of liver injury. With regard to the involvement of the adaptive immune system in DILI, our current understanding is based on the hapten hypothesis and the p-i (pharmacological interaction of drugs with immune receptors) concept. Evidence to support these hypotheses is

gained by the detection of drug-specific antibodies and T cells in some patients with DILI^{1a}.

Berberine is an isoquinoline alkaloid, with a bright yellow color. Berberine is chief alkaloid from roots and stem-bark of *Berberis* species. It is manufactured mostly from roots of *B. aristata* (5% in roots and 4.2% in stem-bark), *B. Petiolaris* (0.43%), *B. vulgaris*^{1b}, *B. aquifolium*, *B. thunbergii* and *B. asiatica*^{2,3} *C. teeta* (rhizome 8-9%) and *Hydrastis Canadensis* Among Chinese herbs, the primary sources are *B. sargentiana*, *Phellodendron amurense* and *Coptis chinensis*, *Coptis chinensis* rhizomes and related species used as its substitutes have about 4–8% berberine, while *Phellodendron amurense* bark has about half as much, at 2–4% berberine. Berberine has molecular formula C₂₀H₁₈NO₄⁺, molar mass 336.36122 g/mol melting point 145° C.

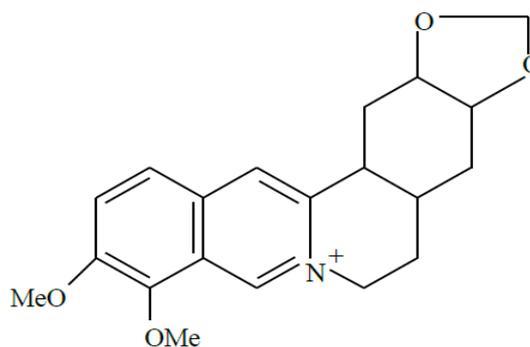


Figure 1.1: Chemical structure of Berberine

Berberine possesses antioxidant, anti-microbial, anti-cancer, induction of cancer cell death, suppression of cancer cell growth, inhibition of cancer cell metastasis, cardiovascular protective effect, anti-atherosclerosis effect, anti-diabetic, neuroprotective effect, anti-obesity, gastrointestinal protective, anti-rheumatic, anti-angiogenic effect, anti-clastogenic effect, anti-convulsant effect, anti-depressant

effect, anti-diarrhea effect, anti-skin aging effect, anti-uveitis effect, muscle-relaxing effect.

Berberine is not considered toxic at doses used in clinical situations, nor has it been shown to be cytotoxic or mutagenic. Side effects can result from high dosages and may include gastrointestinal discomfort, dyspnoea, lowered blood pressure, flu-like symptoms, and cardiac damage. The therapeutic dosage for most clinical situations is 200 mg orally 2 to 4 times daily. The toxicological effects of this compound have been thoroughly studied as follows Oral LD50 (mouse) : >29586 mg/kg, Oral LD50 (rat) : >15000 mg/kg, Intraperitoneal LD50 (mouse): 37 mg/kg and Intravenous LD50 (rat): 60 mg/kg.

Berberine was reported to ameliorate nonalcoholic fatty liver disease (NAFLD). In NAFLD rats, it could improve the recovery of hepatic steatosis and lipid metabolism disorder, and reduce inflammation and insulin resistance, with the mechanism of up-regulation of insulin receptor substrate- 2 (IRS-2) and down-regulation of uncoupling protein-2 (UCP2)⁴. Berberine might also be effective to alcoholic liver disease (ALD). In HepG2 cells, it could inhibit acetaldehyde, the metabolic product of ethanol, induced production of pro-inflammatory factors, such as IL-1 α and TNF- α , probably through NF- κ B signaling pathway. In addition, berberine could inhibit liver fibrosis. In liver fibrosis rodent models, it could protect experimental liver fibrosis through enhancing anti-oxidant system, inhibiting lipid peroxidation and hepatic stellate cell proliferation.

Phytochemical screening is the evidence for the presence of glycoside, saponin and terpenoids in the acetone extract of *T. cordifolia*. Several authors reported Terpenoids in *T. cordifolia*⁵. The petroleum ether extract of *T. cordifolia* also showed the presence of β -sitosterol, octacosanol, heptacosanol⁶. The total phenolic and total tannins were quantitatively estimated in stem parts of *T. cordifolia*⁷. *T. cordifolia* leaves have been investigated, whereby; hexane, chloroform, methanol, ethanol and aqueous extracts were assayed for their total phenolic and flavonoid contents⁸.

Hepatoprotective activity of *Musa paradisiaca* on experimental animal models was studied. Pretreatment with alcoholic extract (500 mg/kg, p.o.), more significantly and to a lesser extent the alcoholic extract (250 mg/kg, p.o.) and aqueous extract (500 mg/kg, p.o.) reduced the elevated level of serum enzymes like SGOT, SGPT, ALP and bilirubin levels. The alcoholic extract at doses of 250 and 500 mg/kg, p.o. and aqueous extract at a dose of 500 mg/kg, p.o. of stem of *M. paradisiaca* have significant effect on the liver of CCl₄ and paracetamol induced hepatotoxicity animal models⁹

Phytochemical analysis, hepatoprotective and antioxidant activity of *Alchornea cordifolia* methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats was evaluated. The degree of protection was measured by using the biochemical parameters such as SGOT, SGPT, ALP, TP and TB. The *in-vitro* antioxidant activity of extract was also evaluated by the DPPH, free radical scavenging assay. The ethyl acetate and chloroform fractions, at a dose of 300 mg/kg, orally produced significant hepatic protection by decreasing the activities of the serum enzymes and bilirubin

while there were marked scavenging of DPPH free radicals by the fractions¹⁰.

Effect of nimesulide on liver functions were analysed by serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and histopathological studies of liver through scoring system. Histopathological variation in liver were observed in therapeutic and supra therapeutic doses in single dose groups and sub therapeutic, therapeutic and supra therapeutic doses in seven days groups. In one dose of nimesulide in litters, there were significant increases in biochemical parameters (p< 0.05) in suprathreshold doses. However, in seven days studies of nimesulide in litters, there were significant increases in biochemical parameters (p< 0.05) in therapeutic and suprathreshold doses. Thus on the basis of observation it can be concluded that nimesulide causes significant hepatotoxicity in litters of rat¹¹.

Nimesulide has been commonly used in pediatric patient for the treatment of inflammation associated to respiratory tract infections, fever, several chronic inflammatory conditions, and pain in many countries. Reactive oxygen species (ROS) has been implicated in nimesulide-induced adverse effects, including hepatotoxicity. However, several reports shown the reducing effect of nimesulide on oxidative damage and its direct free radical scavenging activity. Nimesulide was given by gavage at two doses for 14 days. Blood and tissue samples were taken under pentobarbital anesthesia. Nimesulide treatment caused increase in plasma malondialdehyde (MDA) levels and decrease in catalase (CAT) and glutathione peroxidase (GPx) activities; superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G-6P-DH) activities were not changed. Tissue damage and variation in some serum parameters were also observed. Our results, indicating the possibility of tissue damage and alterations of oxidant/antioxidant status by nimesulide, may provide important contribution to the literature about the restricted use of nimesulide in juveniles¹²

2. Material and Method

2.1 Animal

20 male albino wistar rats, weighing 150-200 gm, were taken from the Central Animal House Facility, I.E.C College of Pharmacy, Greater Noida. The animals were kept in polypropylene cages (5 in each cages) under standard laboratory conditions (12 hr light and 12 hr dark: day: night cycle) and had a free access to commercial pellet diet (Amrut rat feed, Pune, India) and tap water *ad libitum*. The animal house temperature was maintained at 25 + 2^oC and relative humidity was also maintained at (50 + 15 %) as per guidelines of CPCSEA (1332/ac/10/CPCSEA - 30th March 2010). This project was approved by Institutional Animal Ethical Committee

2.2 Drug

Drugs Nimesulide have been purchased from Ranbaxy laboratories limited, Gurgaon, drug Berberine was purchased from Dabar laboratory Ltd, Delhi while LFT Diagnostic Kit were brought from Reckon Diagnostics Pvt.Ltd., Baroda

2.3 Nimesulide induced Hepatotoxicity

Nimesulide at a dose of 100 mg/kg was given for 7 days and after that various parameters was determine in order to ascertain the liver toxicity. finally it was evaluated that animal faced hepatotoxicity. The effect of Beberine at higher and lower dose in this model was assessed by its potency to protect against nimuslide induced hepatotoxicity.

2.4 Experimental design

Animals were divided into 4 groups. Group I received Normal Saline (0.5 ml/kg body wt., p.o.), Group II received Nimesulide drug (Nimesulide, 100 mg/kg body wt., p.o.), Group III received the lower dose of test drug (Berberine 80 mg/kg b.wt. p.o.), Group IV received the higher dose of test drug (Berberine 160 mg/kg body wt., p.o.). After 10 days of drug treatment, on 11th day blood was collected through orbital plexus of rat for estimation of two parameters.

Table: 2.1 Animals required for different groups in Nimesulide induced hepatotoxicity

Groups	Dose (mg/kg)	Animals Required
Normal control	0.5ml/kg normal saline p.o for 7 days	5
Nimesulide	100 mg/kg i.p. for 7 days	5
Nimesulide+(Berberine lower dose)	100 mg/kg i.p.+ 80mg/kg p.o. on days 5, 6, 7, 8, 9 & 10	5
Nimesulide+Berberine (higher dose)	100mg/kg i.p. + 160 mg/kg p.o. on days 5, 6, 7, 8, 9 & 10	5

2.5 Preparation and administration of drug solution

- 1) 0.9% Normal saline: 90 mg sodium chloride was dissolved in 10 ml water for injection.
- 2) Nimesulide solution: The solution was taken directly from the marketed preparation having concentration 2 mg/ml. Having marketed preparation of the doxorubicin hydrochloride (conc. 50 mg/25ml) and 1.25 ml of this solution was given to rats (between 150-200 g) by intraperitoneal route single dose.
- 3) 0.5% Carboxy methylcellulose (CMC): 50 mg carboxy methylcellulose was suspended in 10 ml double distilled water.
- 4) Berberine (Dose: 80 mg/kg): 20 mg of diclofenac sodium was suspended in 10 ml of 0.5% CMC and 1.0 ml of this solution was given to rats (between 150-200 g) by oral route per day.
- 5) Berberine (Dose: 160 mg/kg): oily solution of omega-3 fatty acid directly taken from soft gelatin capsule and given orally to rats as per specified dose.

Animals were divided into 4 groups; each group consisted of 5 animals. The number of groups with their treatment schedule is detailed in Table 2.1. After treating animals as mentioned, the blood samples were withdrawn from the retro-orbital plexus under light anaesthesia for estimation of parameter in serum.

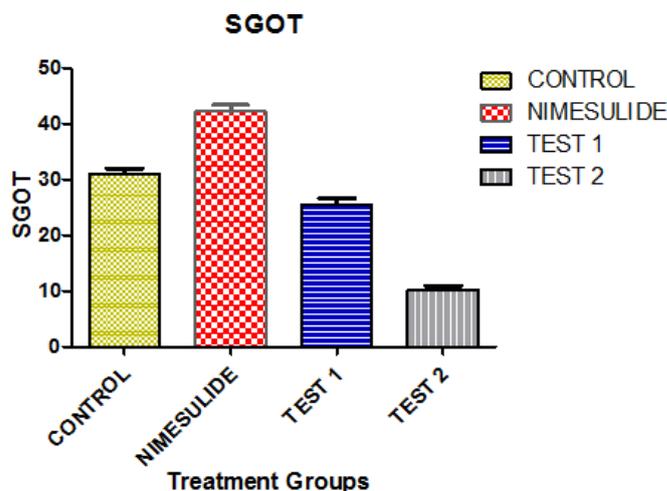
3. Result and Discussion

The measurement of serum SGOT, ALP and levels were used as a biomarker for assessment of liver condition. Nimesulide (100 mg/kg, i.p.) increased serum SGOT, ALP and at the end of 7th day of drug treatment reflecting the liver injury in comparison to control group. Berberine at a dose of 160 mg/kg administered orally for 6 days, from days fifth onwards significantly decreased ($P<0.001$) SGOT, ($P<0.001$) ALP whereas Berberine at a dose of 80 mg/kg showed improvement in ($P<0.01$) SGOT, ($P<0.001$) ALP. Berberine at a dose of 160 mg/kg was more effective than Berberine 80 mg/kg and which alleviated the hepatotoxicity and protect the liver injury caused by Nimesulide. Results are presented in Table 4.1.

Table 3.1: Hepatoprotective effect of berberine on Nimesulide induced Liver toxicity.

Treatment Group	SGOT	ALP
Control	31.00±0.37	52.00±0.53
Nimesulide	42.25±0.42	182±1.14
Berberine (80mg/kg, p.o.)	25.50±0.42**	17.63±0.26***
Berberine (160mg/kg, p.o.)	10.25±0.25***	8.63±0.19***

Statistical significance test was done by ANOVA followed by Tukey's t test (n=5) Values are mean±SEM of 5 animal per group, * $P<0.05$ ** $P<0.01$, *** $P<0.001$ vs. Scopolamine treated group.



Graph: 3.1 SGOT in 4 group of animal

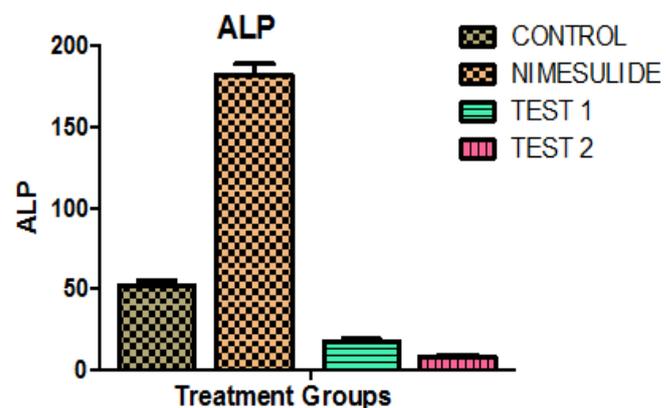


Figure 3.2: ALP in 4 group animal

The liver demonstrates a major role in metabolism of xenobiotics by regulating the synthesis, secretion and metabolism of xenobiotics. Various physiochemical functions of the body including oxidation, reduction, hydroxylation, hydrolysis, conjugation, sulfation, acetylation etc are well balanced by the liver alone.

SGOT (Serum glutamic oxaloacetic transaminase) is an enzyme that is normally present in liver and heart cells. SGOT and SGPT is released into blood when the liver or heart is damaged. The blood SGOT levels are thus elevated with liver damage (for example, from viral hepatitis) or with an insult to the heart (for example, from a heart attack). Some medications can also raise SGOT levels. SGOT is also called aspartate aminotransferase (AST). It facilitates the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate, and vice-versa. It catalyzes the transfer of an amino group from alanine to α -ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate.

The hepatic cells consist of higher concentrations of SGOT in particular exists in mitochondria. Due to the damage caused to hepatic cells, the leakage of plasma causing an increased levels of hepatospecific enzymes in serum. The elevated serum enzyme levels like SGOT are indicative of cellular leakage and functional integrity of cell membrane in liver. The hepatoprotective index of a drug can be evaluated by its capability to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been induced by a hepatotoxin. The measurement of serum SGOT and ALP levels serve as a means for the indirect assessment of condition of liver. Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the Liver.

It has been evaluated that berberine possesses a significant anti-hepatotoxic property. Possible therapeutic mechanism involves inhibition of Phospholipase A₂ and hence production of anti-inflammatory effects, anti-oxidant effect by quenching the free radicals of 1,1-diphenyl-2-picrylhydrazyl (DPPH), attenuation of Nimesulide induced depletion of liver glutathione (GSH), significant membrane stabilizing property and induction high level of DNA repair synthesis (for enhanced proteins synthesis). So its use may be recommended in hepatotoxicities caused by different hepatotoxins. Berberine prevents oxidative damage, as indicated by the decrease in lipid peroxidation, and improves the antioxidant status. Furthermore, berberine suppresses the inflammatory response by downregulating the proinflammatory cascade initiated by TNF- α , and attenuates nitrosative stress by the Inos inhibition.

The present study found that berberine had both preventive and curative effects on Nimesulide - induced liver damage. Moreover, our findings suggest that dosages may be an important factor for curative effects of berberine. The dosage of Berberine (160mg/kg, p.o.) has higher curative and preventive effect than the doses (80mg/kg, p.o.) used in this study. Treatment of berberine significantly decreased serum ALP, and SGOT activities elevated by Nimesulide-induced hepatotoxicity while serum SOD level significantly decreased.

These results demonstrate the preventive hepatoprotective effects of berberine against liver damage induced by Nimesulide.

The effect of Berberine at 160 mg/kg and 80 mg/kg dose were assessed by its potency to protect against Nimesulide induced Hepatotoxicity. At 11th day blood were collected for estimation of various parameters. The measurement of serum SGOT, ALP levels were used as a biomarker for assessment of liver condition. Nimesulide (100 mg/kg, i.p.) increased serum SGOT, ALP at the end of 7th day of drug treatment reflecting the liver injury in comparison to control group. The present study found that berberine had both preventive and curative effects on Nimesulide - induced liver damage. Moreover, our findings suggest that dosages may be an important factor for curative effects of berberine. The dosage of Berberine (160mg/kg, p.o.) has higher curative and preventive effect than the doses (80mg/kg, p.o.) used in this study. Treatment of berberine significantly decreased serum ALP and SGOT activities elevated by Nimesulide-induced hepatotoxicity.

4. Conclusion

The results conclude that hepatoprotective berberine when administered in combination with nimesulide decreases the extent of hepatotoxicity caused by nimesulide alone. So this combination may be the rationale as nimesulide is banned in many country and in India use of this medicine is restricted due to hepatotoxicity. As the nimesulide is better option than many NSAID for its therapeutic use as analgesic, antipyretic and anti inflammatory effect, so the combination of allopathic drug nimesulide with herbal drug berberine prevents its withdrawal from market due to severe hepatotoxicity.

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