



## EVALUATION OF HEPATOPROTECTIVE EFFECT OF BERBERINE IN PARACETAMOL INDUCED EXPERIMENTAL HEPATOTOXICITY IN WISTAR RATS

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### ABSTRACT

Worldwide, liver diseases remain one of the major health problems. Unfortunately, drugs in allopathic medical practices used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. The present study was aimed to evaluate the hepatoprotective activity of berberine, a traditional drug from Southeast Asia, against paracetamol induced liver damage in rats. Paracetamol (2gm/kg) administration to rats resulted in massive elevation in serum liver marker enzymes, serum bilirubin, and thiobarbituric acid reactive substance (TBARS) level with a significant decrease in plasma glutathione (GSH) level, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities. Berberine (120mg/kg) was effectively protecting the animals from hepatotoxicity induced by paracetamol. This was evident from significant reduction in serum liver marker enzymes, serum bilirubin, lipid peroxidation and an increase in plasma SOD, GSH, GPx levels. It was concluded from the result that berberine shows promising hepatoprotective activity against paracetamol induced hepatotoxicity in wistar rats.

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### INTRODUCTION

Liver is the vital organ of metabolism and excretion. About 20,000 deaths found every year due to liver disorders. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2, 50,000 new cases each year (Gupta and Misra, 2006). In India, about 40 polyherbal commercial formulations apparent to have hepatoprotective action are being used. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes (Handa *et al.*, 1986; Sharma *et al.*, 2002). Paracetamol is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses. Paracetamol toxicity is due to the formation of its toxic metabolite, N-acetyl-p-benzo quinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion when a part of it's metabolized by cytochrome P-450. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity (Dahlin *et al.*, 1984; Gupta *et al.*, 2006). The liver is a major organ responsible for the metabolism of drugs and toxic chemicals, and therefore is the primary target organ for nearly all toxic chemicals (Bissell *et al.*, 2001). Natural antioxidants could prevent the deleterious effects of toxic agents by scavenging free radicals and other reactive oxygen species or by modulation of the inflammatory response

(Domitrović *et al.*, 2009). Reactive oxygen free radicals have been known to produce tissue injury through covalent binding and lipid peroxidation. Scavenging of free radicals by antioxidants could reduce the tissue damage. Since the damaging effect of paracetamol is thought to be mainly due to the free radical interaction, it could be worth looking for antioxidants capable of scavenging these reactive species (Domitrović *et al.*, 2011).

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help in regeneration of hepatic cell. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional systems of medicine in India. Berberine is an isoquinoline alkaloid of the protoberberine type, which could be found in the root, rhizome, and stem bark of many plant species traditionally used for treatment of hepatic disorders, such as *Coptis chinensis* Franch., *Coptis japonica* Makino., *Berberis thunbergii* D.C., *Thalictrum lucidum* L., barberry (*Berberis vulgaris* L.), Oregon grape (*Berberis aquifolium* Pursh), and goldenseal (*Hydrastis canadensis* L.) (Imanshahidi and

Hosseinzadeh, 2008). Extensive research within the past decade indicates that berberine possesses a wide range of pharmacological activities, including antioxidative (Rockova *et al.*, 2004), anti-inflammatory (Küpel *et al.*, 2002), and immunoregulative (Kim *et al.*, 2003) activities. Several studies demonstrated the inhibitory effects of berberine on chemically induced cytotoxicity, lipid peroxidation, and oxidative stress in the liver (Hwang *et al.*, 2002; Zhang *et al.*, 2008), including CCl<sub>4</sub>-induced liver damage (Feng *et al.*, 2010). Therefore, the present study was designed to evaluate the hepatoprotective activity of berberine and to study possible mechanism for its hepatoprotective activity through the biochemical parameters against paracetamol induced hepatic damage in wistar rats.

## **MATERIALS AND METHODS**

### **Chemicals**

Acetaminophenone, Berberine (> 95 % purity, CAS NO: 633- 65-8) was purchased from Sigma-Aldrich Chemical Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade, purchased from Hi-media Laboratories Pvt. Ltd., Mumbai, India.

### **Animals**

Albino wistar male rats 7 to 8 weeks old weighing 150-200g were used for the present study. The animals were obtained from central animal house, Rajah Muthiah Institute of Health Sciences, Annamalai University, India and were housed in the Central Animal House with 12 hours light and 12 hours dark cycles. The animals were randomized into experimental and control groups and housed 4 or 5 in a polypropylene cage. Standard pellets obtained from Mysore snack Feed Ltd., Mysore, India were used as a basal diet during the experiment. The control and experimental animals were provided food and drinking water *ad libitum*. The animals were maintained under controlled conditions of temperature ( $27 \pm 2$  °C) and humidity ( $55 \pm 5$  %) with a 12 h light/dark cycle.

### **Paracetamol induced acute hepatotoxicity**

Hepatotoxicity was induced in wistar rats by administering freshly prepared paracetamol (2g/kg body weight) orally as a single dose using an intragastric tube.

### **Experimental design**

The local institutional animal ethics committee (Registration number 160/1999/CPCSEA) of Annamalai University approved the experimental design. Animals were maintained in accordance with the guidelines of ethical committee for animal care of Annamalai University in accordance with Indian National Law on animal care and use.

A total number of 24 rats were divided into 4 groups, each group comprising 6 animals. Group I served as control. Group II rats were similarly treated as group I and on the fifth day paracetamol(2 g/kg, p.o.) was administered. Group III and IV rats were received berberine (120 mg/kg p.o.) for 7 days. On the fifth day paracetamol (2 g/kg, p.o.) was administered, to group III

rats 30 min after the berberine administration. On the seventh day, all the rats were sacrificed under light ether anesthesia; blood and tissue samples were collected and used for the biochemical assays.

### **Biochemical estimations**

One portion of blood samples were collected in sterile centrifuge tube and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min. Another portion of blood samples were collected into heparinised tubes. Plasma was separated by centrifugation at 1000 x g for 15 min. Liver tissues from animals were washed with ice-cold saline and homogenized using appropriate buffer (TBARS, 0.025 M Tris-HCl buffer, pH 7.5; reduced glutathione [GSH] and glutathione peroxidase [GPx], 0.4 M phosphate buffer, pH 7.0; superoxide dismutase [SOD], 0.025 M sodium pyrophosphate buffer, pH 8.3; catalase [CAT], 0.01 M phosphate buffer, pH 7.0) in an all-glass homogenizer with Teflon pestle and used for biochemical estimations.

Serum aspartate transaminase (AST) and alanine transaminase (ALT) were estimated by using 2, 4-dinitrophenyl hydrazine by the method of Mohan and Cook (1957). Serum alkaline phosphatase (ALP) was determined by the method of King and Armstrong (1934). Activity of GGT was assayed by a modified Mesister and Orłowski procedure. The protein content was determined by the method of Lowry *et al.* Lipid peroxidation was estimated as evidenced by the formation of TBARS. TBARS in plasma was assayed by the methods of Yagi. SOD and CAT activities were assessed by the methods of Kakkar *et al* and Sinha respectively. GSH level in the plasma was determined by the method of Beutler and Kelly. Vitamin C and vitamin E levels were assayed by the methods of Omaye *et al* and Desai respectively.

### **Statistical analysis**

The data are expressed as mean  $\pm$  S.D. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) using SPSS version 12.0 for windows (SPSS Inc., Chicago, IL, USA; <http://www.spss.com>). The results were considered statistically significant if the p values were 0.05 or less.

## **RESULTS**

Table 1 shows the body weight and liver weight in control and experimental animals in each group. The mean body weight and liver weight of paracetamol treated rats were significantly decreased as compared to normal rats. Oral administration of berberine to paracetamol treated rats significantly increases their body weight and liver weight as compared to paracetamol treated rats. Control rats treated with berberine alone showed no significant differences in body weight and liver weight as compared to control rats.

The effect of berberine on serum marker enzymes is presented in table 2. The levels of serum AST, ALT, ALP, total bilirubin and GGT were markedly elevated and that of protein decreased in paracetamol

treated animals, indicating liver damage. Administration of berberine at a dose of 120 mg/kg remarkably prevented paracetamol-induced hepatotoxicity in rats.

necrosis (Janbaz and Gilani, 2000; Feng *et al.*, 2010). Paracetamol administration increased plasma lipid peroxides significantly. Berberine treatment could reduce

**Table 1** Body weight and liver weight of normal and experimental animals in each group

Groups	Body weight (g)		Liver weight (g)
	Initial	Final	
Normal	184.2 ± 10.5 <sup>a</sup>	221.3 ± 12.3 <sup>a</sup>	5.74 ± 0.4 <sup>a</sup>
Paracetamol	182.9 ± 9.8 <sup>b</sup>	166.4 ± 12.4 <sup>b</sup>	5.4 ± 0.3 <sup>b</sup>
Paracetamol + Berberine (120mg/kg bw po)	186.4 ± 9.6 <sup>c</sup>	212.6 ± 9.8 <sup>c</sup>	5.68 ± 0.4 <sup>c</sup>
Berberine alone	183.7 ± 10.3 <sup>a</sup>	220.5 ± 12.1 <sup>a</sup>	5.72 ± 0.4 <sup>a</sup>

Values are given as mean ± SD (n=6)

Values not sharing a common superscript letter significantly at p<0.05 (DMRT)

**Table 2** Activities of liver marker enzymes and bilirubin level in control and experimental animals in each group

Groups	AST (IU/dl)	ALT (IU/dl)	ALP (IU/dl)	GGT (IU/dl)	Bilirubin (mg/dl)
Normal	82.7 ± 6.1	35.2 ± 2.7	76.4 ± 6.4	1.98 ± 0.14	0.65 ± 0.06
Paracetamol	112.4 ± 10.5	69.4 ± 6.4	118.6 ± 9.4	5.25 ± 0.53	1.62 ± 0.13
Paracetamol + Berberine (120mg/kg bw po)	85.2 ± 9.2	38.5 ± 3.1	84.5 ± 6.9	2.24 ± 0.27	0.83 ± 0.07
Berberine alone	81.5 ± 6.2	34.8 ± 2.9	95.8 ± 6.3	2.02 ± 0.15	0.64 ± 0.05

**Table 3** TBARS level, enzymatic and non-enzymatic antioxidants activities in plasma of normal and experimental animals in each group

Groups	TBARS (nmol/ml)	SOD (U <sup>A</sup> /ml)	CAT (U <sup>B</sup> /ml)	GSH (mg/dl)	Vitamin E (mg/dl)	Vitamin C (mg/dl)
Normal	1.82 ± 0.07	4.24 ± 0.34	0.76 ± 0.06	25.4 ± 2.2	1.28 ± 0.11	1.29 ± 0.12
Paracetamol	3.05 ± 0.25	2.84 ± 0.22	0.52 ± 0.05	17.8 ± 1.8	0.72 ± 0.06	0.68 ± 0.07
Paracetamol + Berberine (120mg/kg bw po)	1.98 ± 0.12	4.06 ± 0.52	0.69 ± 0.04	23.7 ± 2.1	1.17 ± 0.08	1.18 ± 0.08
Berberine alone	1.81 ± 0.15	4.25 ± 0.32	0.75 ± 0.07	25.1 ± 2.4	1.29 ± 0.12	1.30 ± 0.11

Values are given as mean ± SD (n=6)

Values not sharing a common superscript letter significantly at p<0.05 (DMRT)

A: The amount of enzyme required to inhibit 50% NBT reduction

B: Micromoles of H<sub>2</sub>O<sub>2</sub> utilized/sec

Table 3 shows the level of plasma TBARS, enzymatic antioxidants (SOD, CAT and GPx) and non-enzymatic antioxidants (Vitamin C, Vitamin E and Glutathione). The levels of TBARS were significantly increased in paracetamol treated rats as compared to control animals. Oral administration of berberine to paracetamol treated rats revert back the levels of TBARS to near normal range. The levels of GSH, Vitamin C and E and activities of SOD and CAT were significantly decreased in paracetamol toxicated rats as compared to control animals. Oral administration of berberine to paracetamol treated rats brought back the level of reduced glutathione, Vitamin C and E and enzymatic antioxidants activities to near normal. Animals treated with berberine alone showed no significant differences in level of TBARS, reduced glutathione, Vitamin C and E and enzymatic antioxidant activities as compared to control rats.

## DISCUSSION

The results of the present study demonstrated that the various biochemical changes produced in paracetamol toxicity were prevented by berberine treatment. The toxicities produced by certain hepatotoxins and carcinogens have been postulated to be due to the formation of chemically reactive metabolic products. Free radical mediated reactions are involved in the inflammatory response which can contribute to liver

these elevated levels. Acetaminophen (Paracetamol) is an extensively used antipyretic-analgesic drug produces acute hepatic damage on over dosage. It is established that, a fraction of acetaminophen is converted via the cytochrome P450 pathway to a highly toxic metabolite; N-acetyl-p-benzoquinamine (NAPQI) (Dahlin *et al.*, 1984) which is usually conjugated with glutathione and excreted in urine. Overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, results in mitochondrial dysfunction (Gupta and Misra, 2006) and the development of acute hepatic necrosis. Several P450 enzymes are known to take part in an important role in acetaminophen bioactivation to NAPQI. P450 2E1 have been suggested to be primary enzyme for acetaminophen bioactivation in liver microsomes. Studies demonstrated that acetaminophen induced hepatotoxicity can be modulated by substances that influence P450 activity (Rajesh *et al.*, 2009).

In the assessment of liver damage by acetaminophen the determination of enzyme levels such as AST, ALT is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. A high level of AST indicates liver damage. 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. Elevated levels of

serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Domitrović, 2011). Serum ALP, bilirubin and total protein levels 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 (Muriel and Garcipiana, 1992).

The abnormal high level of serum ALT, AST, ALP, GGT and bilirubin observed in our study are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with berberine reduced the enhanced level of serum ALT, AST, ALP GGT and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells. There was a significant decrease of these enzyme levels on administration of berberine 120mg/kg bw po. The reversal of increased serum enzymes in acetaminophen-induced liver damage by berberine may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to near normal. Effective control of ALP and bilirubin levels points towards an early improvement in the secretory mechanism of the hepatic cells. The efficacy of any hepatoprotective drug is dependent on its capacity of reducing the harmful effect induced by hepatotoxin. The observed hepatoprotective activity of berberine may be due to its role on modulating liver marker enzymes. Further studies need to elucidate the mechanism are in progress.

The present study indicated that berberine decreased the lipid peroxides level induced by paracetamol treatment. Berberine (120 mg/kg bw) could produce a significant reduction to these elevated levels. Plasma antioxidants level also showed significant alteration, when paracetamol was administered. Simultaneous administration of berberine could reverse these altered values to near normal significantly. Antioxidants such as vitamin E, ellagic acid and curcumin have been reported to protect from liver fibrosis induced by hepatotoxins (Nishigaki *et al.*, 1992; Thressiamma, K.C., Kuttan, 1996). *Berberis aristata* from which berberine isolated is used in Chinese medicine as well as in much Indian medicinal preparation as a hepatoprotector (Antarkar *et al.*, 1980). Moreover berberine was able to scavenge the free radicals generated *in vitro* (Aoxue Luo and Yijun Fan, 2011). The results of the present study indicate the partial hepatoprotective activity of berberine against the paracetamol induced hepatotoxicity.

From our results, it can be speculated that (i) decreasing effect of GSH, SOD, CAT, vitamin C and vitamin E and increasing effect of thiobarbituric acid reactive substance level in rats treated with paracetamol were due to hepatocellular damage and (ii) Berberine afforded protection from such paracetamol induced liver damage. Possible mechanism that may be responsible for the protection of paracetamol induced the following berberine by it self-act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. Its ability is to

inhibit lipid peroxidation and to scavenge on radicals. Thus, by trapping oxygen related free radicals berberine could hinder their interaction with polyester fatty acids and would abolish the enhancement of lipid peroxidative processes. Berberine pretreatment exhibited a normal effect on the GSH, SOD, CAT, vitamin C and vitamin E in blood. Berberine significantly increased blood glutathione. The present results suggested that significantly higher glutathione content, SOD, CAT, vitamin C and vitamin E in blood would offer a better protection against an oxidative stress, thus contributing to the abolishment of paracetamol infused hepatotoxicity. Therefore, berberine may be useful agent for the normalization of paracetamol induced impairments.

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