FREE RADICAL SCAVENGING ACTIVITY OF BERBERINE IN ACETAMINOPHEN INDUCED LIVER INJURY

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ABSTRACT

Objective: Evaluation of free radical scavenging activity of Berberine (BBR) in acetaminophen (AAP) induced liver injury. **Study design:** Experimental study. **Place and Duration:** Animal House, Isra University Hyderabad from October 2015 to March 2016. **Methodology:** A sample of 80 male Wistar rats was selected according to inclusion and exclusion criteria and was divided into a control and three experimental groups. Acetaminophen, N-acetyl cysteine (NAC) and BBR were administered in standard doses. Cardiac puncture collected blood samples after 18 hours of the post-experiment period. Liver function test, antioxidant enzymes, and malondialdehyde (MDA) were detected by ELISA assay kit (Fortress Diagnostics). The data was analyzed on Statistix 10.0 software (USA) at 95% CI ($P \leq 0.05$). **Results:** The BBR showed antioxidant and antiperoxidant activity against acetaminophen-induced liver injury. BBR treated animals showed increased serum and tissue SOD, GPX, CAT, and GSSH with a reduction in tissue MDA (p=0.0001). Liver injury ameliorating effect of BBR was superior to N-acetyl cysteine. **Conclusion:** The present study suggests Berberine protects against acetaminophen-induced liver injury by its free radical scavenging activity.

KEYWORDS: Berberine, N-acetyl cysteine, Acetaminophen, Free radical

Introduction

Drug-induced liver injury (DILI), such as that caused by the acetaminophen, is not an uncommon toxicological problem encountered in the medical practice. In the case of severe toxicity, this results in severe liver injury, hepatocellular necrosis, and death. The liver microsome detoxifies many drugs and toxic agents. [1] Acute fulminant liver failure is the end results with hepatic encephalopathy, coagulopathies, electrolyte and acid-base imbalance and death. Acetaminophen (AAP) is used as an analgesic and antipyretic drug because of easy availability. It is an over-the-counter (OTC) product available in several formulations. [1,2] In United States, over 60 million people use the AAP on a regular basis. [3] While its use in the developing countries is not registered. AAP is approved by FDA as safe in doses up to 4000 mg in 24 hours, so generally it is considered safe and nontoxic at regular doses. [4,5] AAP is available in various formulations such as the liquid syrup, tablets, infusions, rectal suppositories and combination supplements as OTC. [3] The reactive oxygen species (ROS) generated by drug toxicity, are neutralized by in-built enzyme systems of the liver; such as the superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and reduced glutathione (GSSH), and others. N-acetylcysteine (NAC) is a thiol-containing agent used since

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three decades as an antidote for AAP toxicity. [5,6] Clinical efficacy of NAC is well established. Clinically it is utilized for the chronic obstructive pulmonary disease (COPD) and contrast-induced nephropathy. [6-8] Nowadays a growing interest has been found in herbal agents against DILI. Berberine (BBR) is one of herbal derivative used as OTC drug in China. The BBR is a plant isoquinoline alkaloid, traditionally used to treat infectious diarrhea. [10] The BBR is derived from the Rhizoma Coptidis Franch which is an indigenous Chinese herb. Rhizoma Coptidis Franch contains 5.2%–7.7% of berberine. It has been used for soothing of inflammatory reactions and treatment of diabetes mellitus (DM), and others. [10-12] Pleiotropic clinical effects of BBR have been reported such as use in liver disease, obesity [13-15], dyslipidemia, and cardiac disorders, and others. [16,17] The present study evaluated the free radical scavenging activity of Berberine in acetaminophen-induced liver injury in comparison to N-acetyl cysteine.

MATERIALS AND METHODS

The present experimental study was conducted, comprising a sample of 80 male Wistar rats, at the animal house of Isra University Hyderabad, Sindh. The study covered duration from October 2015 to March 2016. Animals belonged to the species produced originally by Charles River Breeding Laboratories, Brooklyn, Massachusetts, USA. They were housed at the Basic Medical Sciences Institute (BMSI) animal house of Jinnah Postgraduate Medical Center (JPMC). The animals were acclimatized for ten days at our animal house which is equipped with modern stainless steel cages with automatic parts for feeding and water drinking.

Animal housing

Animals housing was by the ethics guidelines of NIH and Ethics guidelines of the institute. Animals were acclimatized for ten days before the experiment. The animal was kept in stainless steel cages, which are equipped with automatic parts. The temperature was maintained at 23 ± 2°C. Dark – light cycles of 12 hours were strictly maintained for the experimental animals. We provide pellet diet and fresh clean water. Access to food and water was ad libitum.

Inclusion and exclusion criteria

Adult male Wistar rats of 8-12 weeks age of 170-200 grams were included. Sick, lazy male rats and female rats were the exclusion criteria.

Experimental design

80 male Wistar rats were randomly divided into a control and 3 experimental groups; Group A (n=20): Normal healthy rats taken as controls (0.9% saline water) Group B (n=20): Acetaminophen induced liver injury (2gram/kg bwt) daily [18] Group C (n=20): Acetaminophen (2gram/kg bwt) daily + N-acetyl cysteine (100 mg/kg bwt) for 21 days [5] Group D (n=20): Acetaminophen (2gram/kg bwt) daily + Berberine (100 mg/kg bwt) for 21 days. [19]

Drugs and Chemicals

Acetaminophen (Panadol ®) was purchased from Glaxo Smith Kline, Pakistan. Berberine hydrochloride was purchased from China by Pharmacy Department. "Tweens 80" was purchased. It was used for formulation preparation in sterile H2O.

Berberine toxicity

To test the acute toxicity of Berberine hydrochloride, four rat groups were used; six rats in each group. Berberine hydrochloride was suspended in distilled water. It was given orally by gavages once daily for seven days at 200, 500, 1000, and 2000mg/kg doses. Rats were observed continuously on the first day and then twice for seven days. Toxicity was not observed up to maximum dose of 2000mg/kg. [19]

Blood sampling

Disposable syringes (BD, USA) were used for blood sampling by cardiac puncture after 18 hours of the post-experiment period. Blood samples were collected in gel tubes. Samples were incubated at 4000 rpm for 10 minutes to separate sera. The sera were stored at -200C for biochemical analysis. Any serum sample contaminated with blood was discarded immediately. [20]

Biochemical testing

Liver function tests were performed spectrophotometrically using a commercial kit by standard methods on Roche Biochemical analyzer (Cobas e 411 analyzers, Roche Diagnostics GmbH, Mannheim, Germany). For Prothrombin time, a blood sample was taken in coagulation tubes and estimated by Humaclot duo using standard reagent kits of Merck. [21] Serum creatinine was measured by colorimetric Jaffé method. Superoxide dismutase, glutathione peroxidase, catalase, reduced glutathione (GSSH), and malondialdehyde (MDA) were detected by ELISA assay kit (Fortress Diagnostics) using standard methods mentioned in the literature. [22- 24]

Statistical analysis

The data was analyzed on Statistix 10.0 software (USA). One-way analysis of variance (ANOVA) was used for group comparisons followed by Bonferroni's test for multiple comparisons. Data was presented as mean, SD, and SEM. The confidence interval of statistical significance was defined at 95% ($P \leq 0.05$).

Results

Alanine transaminase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase, -glutamyl transferase, serum bilirubin and Prothrombin time (PT) are shown in Table 1. Escape special TeX symbols (Compress whitespace Escape special TeX symbols (Compress whitespace

Acetaminophen treated group B showed major rises in liver aminotransferases. NAC and BBR treated groups showed amelioration of cytoplasmic and mitochondrial enzymes. Amelioration of liver injury markers was more efficient in BBR treated animals than the NAC group ($p = 0.0001$). Markers of oxidative stress and lipid peroxidation were severely raised in acetaminophen-treated animals (group B). Group B animals showed elevated tissue MDA levels with a significant decrease in the SOD, GPX, CAT and GSSH compared to controls ($p=0.0001$). Markers of oxidative stress and lipid peroxidation of acetaminophen-induced liver injury were found significantly improved in rats treated with NAC and BBR drugs. The serum SOD, GPX, CAT, Scr and tissue SOD, GPX, CAT, GSSH, and tissue MDA showed statistically significant improvement as shown in Tables 2 and 3. Escape special TeX symbols (Compress whitespace

A rise in serum SOD, GPX, CAT and tissue SOD, GPX, CAT, GSSH with a decrease in MDA was noted in NAC, and BBR

Table 1 Liver function tests of experimental rats (n=80)

		Mean	SD	SEM	95% Confidence Interval for Mean		P-value
					Lower boundary	Upper boundary	
Alanine transaminase(U/L)	**Group. A Control**	33.90	6.93	1.55	30.66	37.14	0.0001
	Group B. AAP	70.85	14.09	3.15	64.25	77.45	
	Group C. AAP+ NAC	59.05	9.26	2.07	54.72	63.38	
	Group D. AAP+ BBR	54.10	8.67	1.94	50.04	58.16	
Aspartate transaminase(U/L)	**Group. A Control**	32.50	5.58	1.25	29.89	35.11	0.0003
	Group B. AAP	43.50	20.31	4.54	34.00	53.00	
	Group C. AAP+ NAC	37.55	11.78	2.63	32.04	43.06	
	Group D. AAP+ BBR	35.05	9.20	2.06	30.75	39.35	
Alkaline Phosphatase (U/L)	**Group. A Control**	81.55	19.40	4.34	72.47	90.63	0.0001
	Group B. AAP	141.60	31.45	7.03	126.88	156.32	
	Group C. AAP+ NAC	105.80	44.67	9.99	84.89	126.71	
	Group D. AAP+ BBR	96.40	34.94	7.81	80.05	112.75	
Lactate Dehydrogenase (U/L)	**Group. A Control**	110.10	17.67	3.95	101.83	118.37	0.0001
	Group B. AAP	165.50	32.02	7.16	150.52	180.48	
	Group C. AAP+ NAC	143.55	33.16	7.41	128.03	159.07	
	Group D. AAP+ BBR	131.50	24.86	5.56	119.87	143.13	
γ -Glutamyl transferase(U/L)	**Group. A Control**	34.45	5.78	1.29	31.74	37.16	0.0003
	Group B. AAP	72.05	18.57	4.15	63.36	80.74	
	Group C. AAP+ NAC	56.65	19.88	4.45	47.34	65.96	
	Group D. AAP+ BBR	49.85	22.42	5.01	39.36	60.34	
S. Bilirubin (mg/dL)	**Group. A Control**	0.59	0.13	0.03	0.53	0.65	0.0002
	Group B. AAP	2.45	0.82	0.18	2.06	2.83	
	Group C. AAP+ NAC	1.70	1.04	0.23	1.21	2.18	
	Group D. AAP+ BBR	1.27	0.38	0.08	1.10	1.45	
Prothrombin time (sec)	**Group. A Control**	9.29	1.94	0.43	8.38	10.19	0.0001
	Group B. AAP	14.80	1.19	0.27	14.25	15.36	
	Group C. AAP+ NAC	11.77	3.27	0.73	10.24	13.30	
	Group D. AAP+ BBR	9.92	2.64	0.59	8.68	11.15	
AAP – acetaminophen, NA- N-acetyl cysteine, BBR – Berberine							

Table 2 Serum superoxide dismutase, glutathione peroxidase, catalase and creatinine (n=80)

		Mean	SD	SEM	95% Confidence Interval for Mean		P-value
					Lower boundary	Upper boundary	
Serum SOD (U/ml)	**Group. A Control**	135.65	35.70	7.98	118.94	152.36	0.0003
	Group B. AAP	77.45	17.54	3.92	69.24	85.66	
	Group C. AAP+ NAC	111.80	20.71	4.63	102.11	121.49	
	Group D. AAP+ BBR	123.85	14.51	3.24	117.06	130.64	
Serum GPX (nM/min/mL)	**Group. A Control**	135.50	36.84	8.24	118.26	152.74	0.0002
	Group B. AAP	89.05	24.15	5.40	77.75	100.35	
	Group C. AAP+ NAC	119.10	18.87	4.22	110.27	127.93	
	Group D. AAP+ BBR	125.25	7.09	1.58	121.93	128.57	
Serum CAT (nM/min/mL)	**Group. A Control**	408.55	80.13	17.92	371.05	446.05	0.0007
	Group B. AAP	173.80	102.43	22.90	125.86	221.74	
	Group C. AAP+ NAC	191.45	117.09	26.18	136.65	246.25	
	Group D. AAP+ BBR	266.45	132.55	29.64	204.42	328.48	
Serum Creatinine (Scr) (mg/dl)	**Group. A Control**	1.49	0.73	0.16	1.15	1.83	0.0001
	Group B. AAP	3.73	1.10	0.25	3.21	4.24	
	Group C. AAP+ NAC	1.70	1.04	0.23	1.21	2.18	
	Group D. AAP+ BBR	1.27	.38	0.08	1.10	1.45	
AAP – acetaminophen, NA- N-acetyl cysteine, BBR – Berberine							

Table 3 Liver tissue anti oxidant and lipid peroxidant markers (n=80)

		Mean	SD	SEM	95% Confidence Interval for Mean		P-value
					Lower boundary	Upper boundary	
Tissue SOD (U/ml)	**Group. A Control**	134.80	45.17	10.10	113.66	155.94	0.0003
	Group B. AAP	86.40	6.10	1.36	83.55	89.25	
	Group C. AAP+ NAC	126.45	24.38	5.45	115.04	137.86	
	Group D. AAP+ BBR	131.20	31.76	7.10	116.34	146.06	
Tissue GPX (nM/min/mg)	**Group. A Control**	149.70	48.21	10.78	127.14	172.26	0.0002
	Group B. AAP	82.80	21.72	4.86	72.63	92.97	
	Group C. AAP+ NAC	105.75	38.80	8.68	87.59	123.91	
	Group D. AAP+ BBR	118.55	29.76	6.66	104.62	132.48	
Tissue CAT (nM/min/mg)	**Group. A Control**	299.10	34.08	7.62	283.15	315.05	0.0007
	Group B. AAP	116.70	61.70	13.80	87.82	145.58	
	Group C. AAP+ NAC	187.20	120.50	26.94	130.80	243.60	
	Group D. AAP+ BBR	239.20	125.37	28.03	180.53	297.87	
Tissue GSH (µM/mg)	**Group. A Control**	3.91	0.34	0.08	3.75	4.06	0.0001
	Group B. AAP	1.96	0.52	0.12	1.71	2.20	
	Group C. AAP+ NAC	2.82	0.93	0.21	2.39	3.26	
	Group D. AAP+ BBR	3.17	0.84	0.19	2.77	3.56	
Tissue Malondialdehyde(µmol/gWTW)	**Group. A Control**	3.05	1.45	0.32	2.37	3.72	0.0001
	Group B. AAP	6.72	2.85	0.64	5.39	8.05	
	Group C. AAP+ NAC	5.10	1.72	0.38	4.30	5.91	
	Group D. AAP+ BBR	3.70	1.90	0.42	2.82	4.59	
AAP – acetaminophen, NA- N-acetyl cysteine, BBR – Berberine							

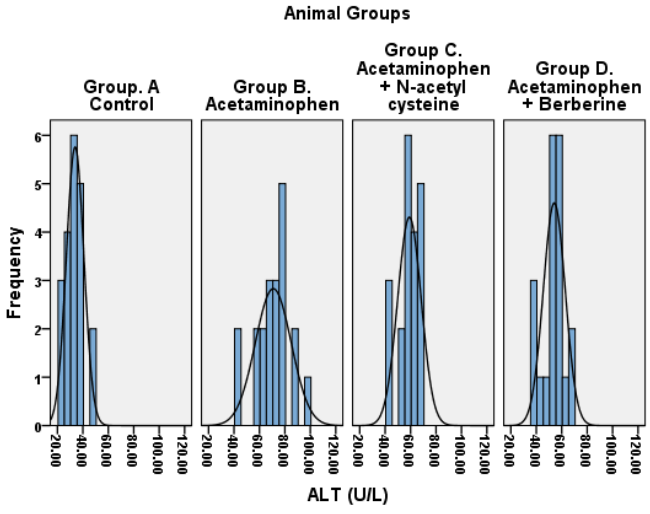

Figure 1: Serum Alanine transaminase

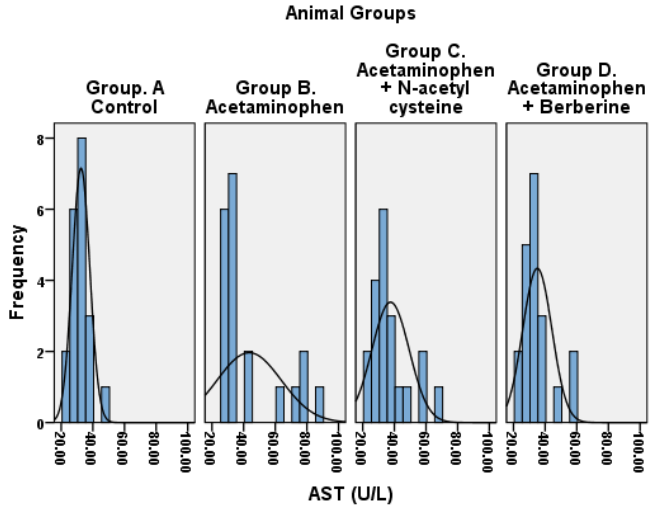

Figure 2: Serum aspartate transaminase

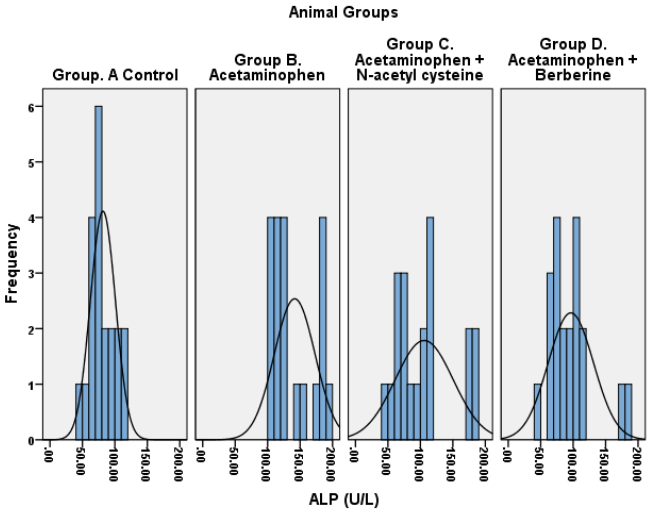

Figure 3: Serum Alkaline phosphatase

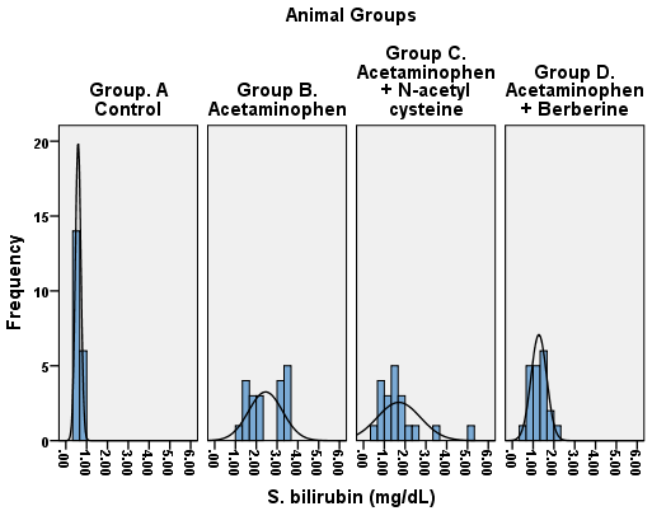

Figure 6: Serum bilirubin

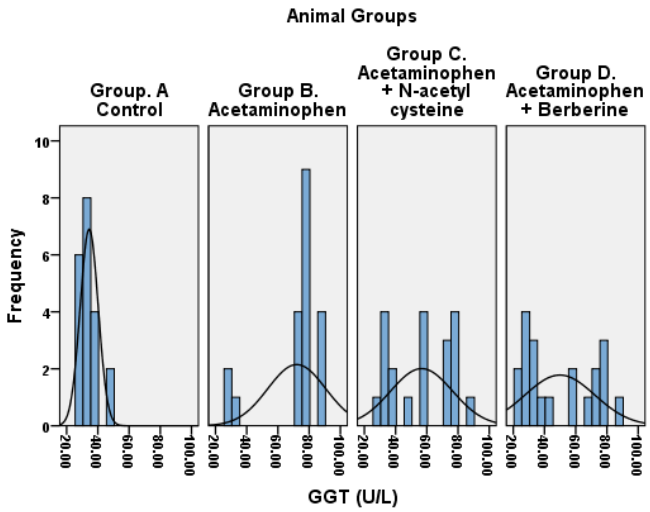

Figure 4: Serum γ-glutamyl transferase

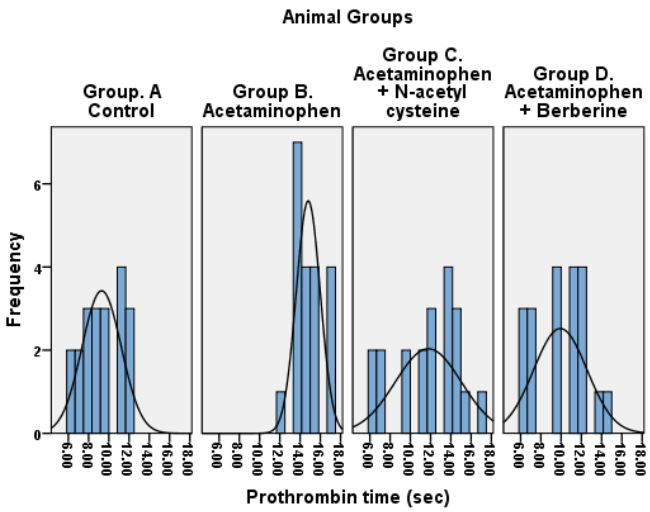

Figure 7: Prothrombin time

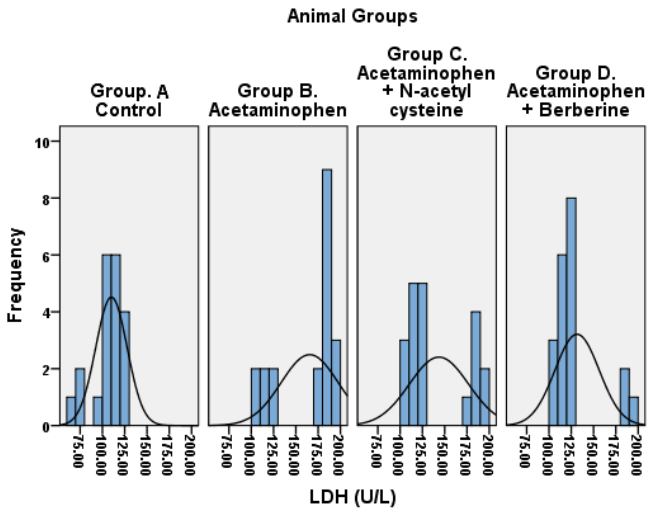

Figure 5: Serum Lactate dehydrogenase

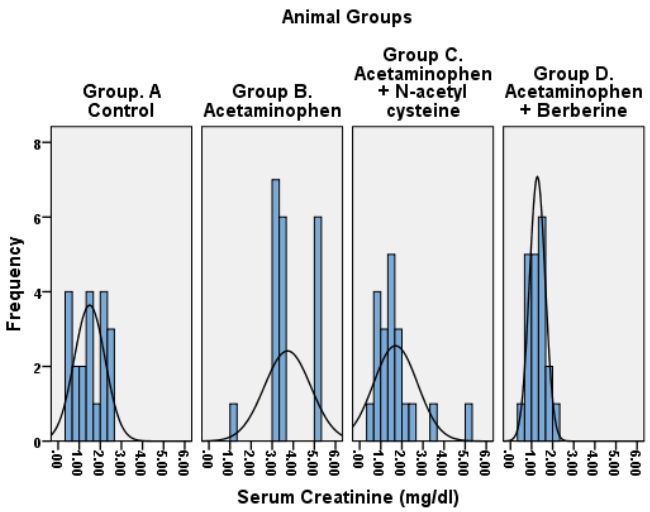

Figure 8: Serum creatinine

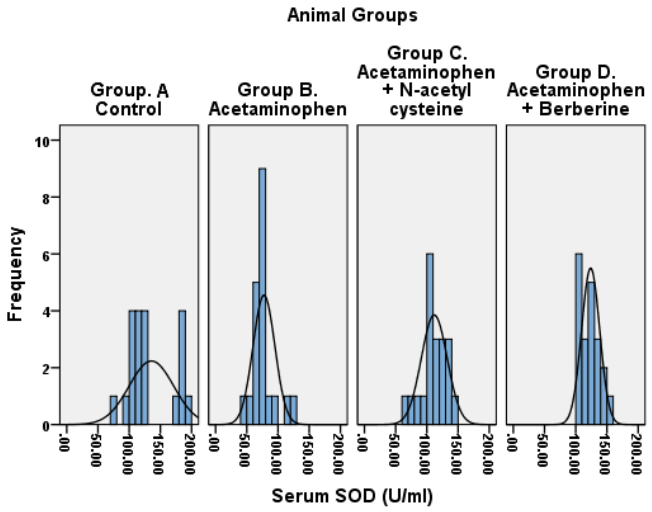

Figure 9: Serum Superoxide dismutase

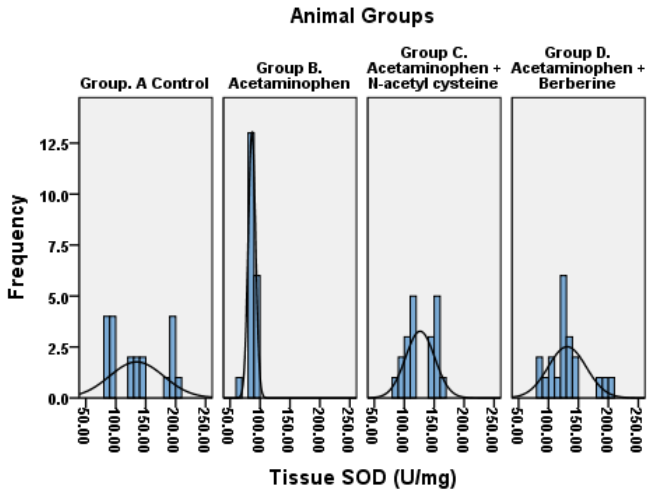

Figure 12: Tissue superoxide dismutase levels

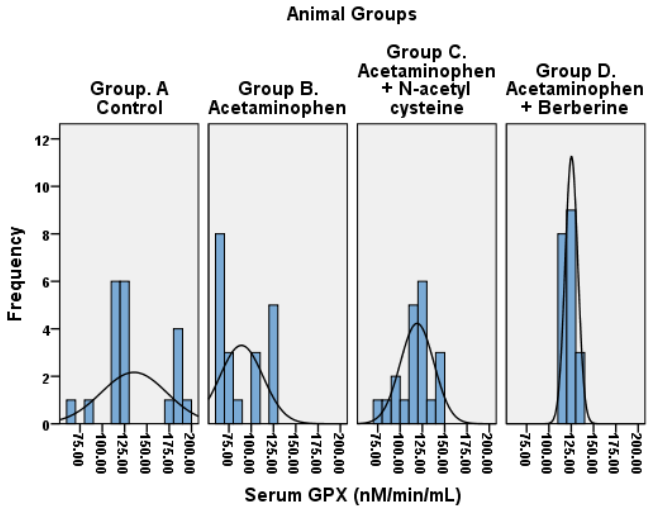

Figure 10: Serum Glutathione peroxidase

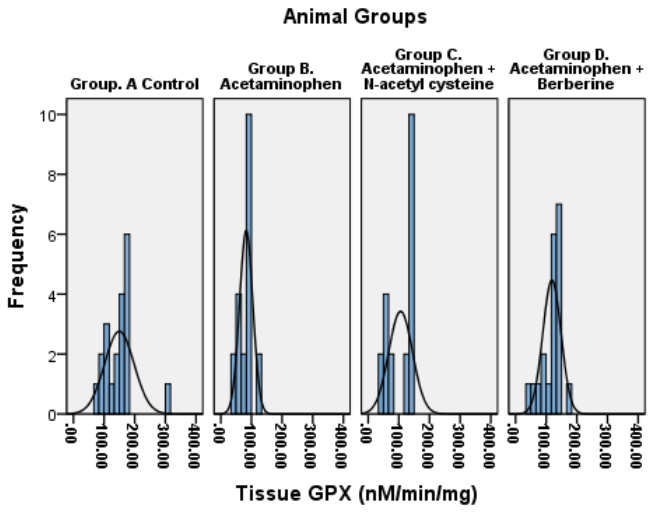

Figure 13: Tissue Glutathione peroxidase levels

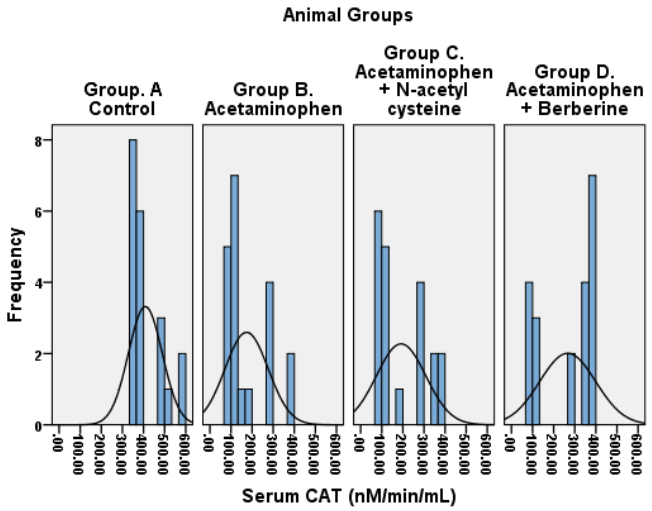

Figure 11: Serum Catalase

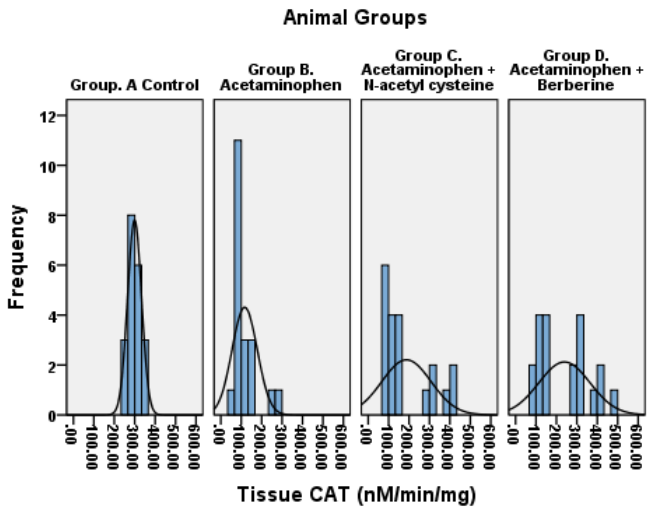

Figure 14: Tissue Catalase levels

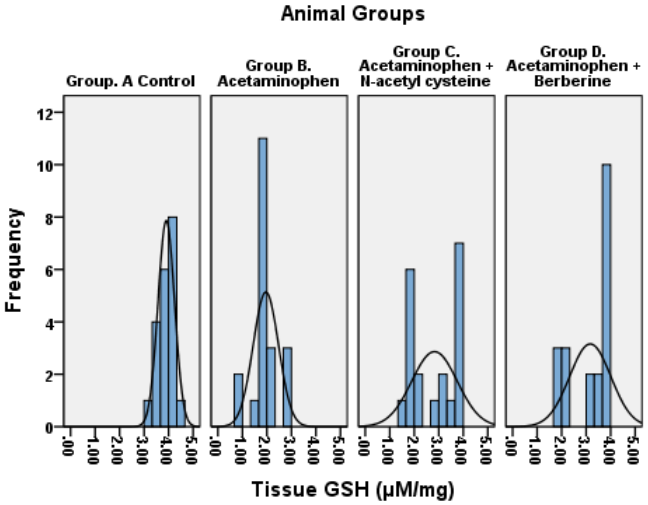

Figure 15: Tissue Reduced Glutathione levels

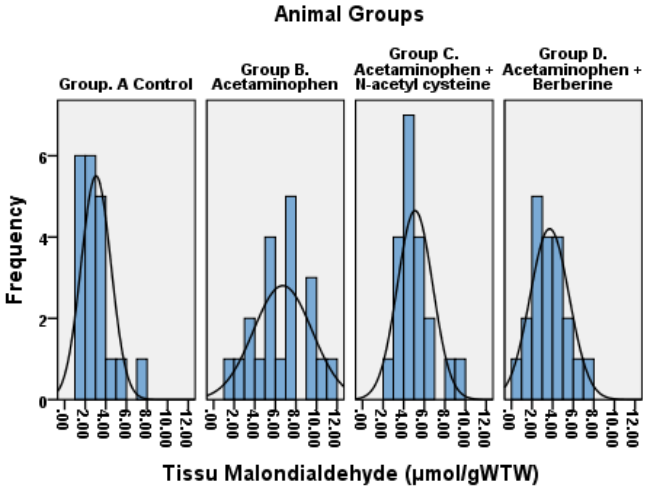

Figure 16: Tissue Malondialdehyde

treated rats. The improvement of liver injury was significantly high in BBR group compared to other groups (p=0.0001).

Graphs 1-16 show the distribution of antioxidant enzymes, serum creatinine, and MDA. Acetaminophen-induced oxidative stress and lipid peroxidation revealed amelioration in the BBR and NAC-treated rats. Berberine response of ameliorating liver injury was superior to N-acetyl cysteine.

Discussion

Acetaminophen (AAP) induced liver toxicity was first reported in the United States in the mid-1980s, since then a rising trend has been reported from the World. AAP is one of the most commonly used drugs implicated in DILI. [1,3,4] A mortality of 0.4% has been reported, approximating 300 deaths per year in the USA. [2] AAP-induced liver injury is mediated through production of reactive oxygen species (ROS). [1,25,26] AAP, when taken in megadoses, is metabolized into N-acetyl p-benzoquinoneimine (NAPQI). The NAPQI is a highly reactive agent which induces liver injury by free radical formation. [27,28] AAP induces oxidative stress and lipid peroxidation and depletes the liver of GSSH. [27-30] In the present study, the Berberine was evaluated for its free radical scavenging activity in comparison to a standard drug; the N-acetyl cysteine (NAC). In our current study the hepatotoxicity due to acetaminophen was confirmed by elevated levels of biochemical parameters; like ALT, AST, ALP,γ -GT, PT and serum bilirubin in the experimental group. Acetaminophen treated group B showed a relentless rise in liver aminotransferases. The NAC and BBR treated groups showed amelioration of cytoplasmic and mitochondrial enzymes. The BBR treated animals showed more significant amelioration compared to NAC group (p = 0.0001). In the case of liver cell injury, the cytoplasmic and mitochondrial enzymes leak through the cell membrane, [31] and rise in the blood. [32] Treatment with NAC and BBR showed decreased levels of biochemical parameters; such as ALT, AST, ALP, γ -GT, PT, and serum bilirubin. This may be due to free radical scavenging activity of BBR and stabilization of cell membrane. Previous studies [30-34] supports the mechanisms. Serum ALP rises due to increased synthesis by the biliary canaliculi in response to biliary pressure because of cholestasis.[33,34] The present study reports free radical scavenging activity of BBR and NAC as evaluated by markers of oxidation and peroxidation. The BBR treated rats showed a statistically significant rise in serum and tissue SOD, GPX, CAT and GSSH and a decline in MDA. Antioxidant and anti-lipid peroxidant effect of BBR was superior to the NAC. Our findings are in agreement with the previous studies. [35,36] It is reported that the rise in MDA indicates failure of cellular antioxidant enzymes. [37] The BBR and NAC-treated rats showed a significant decrease in MDA; this suggests they possess antioxidant and anti peroxidant potential (Table 3). The BBR prevents hepatocyte cell injury by scavenging free radicals through an increase in cellular antioxidants – the SOD, GPX, CAT, and GSSH. The findings are in agreement with previous studies. [38,39] The berberine mitigates the oxidative injury by raising the SOD, GPX, CAT and GSSH which is visibly evident in the present study.

Conclusion

The present study suggests a significant hepatoprotective effect of Berberine against acetaminophen-induced liver injury. The Berberine showed antioxidant and anti peroxidant potential.

Berberine increases the antioxidant enzymes activity – the superoxide dismutase, glutathione peroxidase, catalase and reduced glutathione, and decreases the malondialdehyde. The findings point towards free radical scavenging activity of Berberine.

Authors' Statements

Competing Interests

The authors declare no conflict of interest.

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Source of Support:

None

Conflict of Interest:

None

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