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In vitro antioxidant activity and *in vivo* hepatoprotective activity of aqueous extract of *Allium cepa* bulb in paracetamol induced liver damage in Wistar rats

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ABSTRACT

The *in vitro* antioxidant and *in vivo* hepatoprotective effects of aqueous extract of *Allium cepa* bulb were evaluated in male Wistar rats against paracetamol induced liver damage in preventive and curative models. The antioxidant activity of *Allium cepa* was assayed by DPPH, hydroxyl and superoxide radical scavenging activity. The various antioxidant activities were compared to standard antioxidant, ascorbic acid. In two different set of experiments, the *Allium cepa* extracts (100,300 and 600 mg/kg body weight (bw), orally) and silymarin (100 mg/kg bw, orally) were administered orally in both the studies. Liver injury was induced by paracetamol administration (2 g/kg bw, orally). In the 2,2-diphenyl-1-picrylhydrazil (DPPH), hydroxyl and superoxide radical scavenging activity, the IC_{50} values of aqueous extract was 195.2 ± 0.2, 374.7 ± 0.4 and 182.5 ± 1.7 µg/ml respectively. The level of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were determined to assay hepatotoxicity. Paracetamol administration caused severe hepatic damage in rats as evidenced by elevated serum AST, ALT, ALP and total bilirubin levels. The *Allium cepa* and silymarin administration prevented the toxic effect of paracetamol on the above serum parameters in both preventive and curative models. The present study concludes that aqueous extract of *Allium cepa* bulb has significant antioxidant and hepatoprotective activity against paracetamol induced hepatotoxicity, which may be associated with its high bioactive compounds including flavonoids, phenolic compound, pectic polysaccharide and antioxidant properties.
Key words: *Allium cepa,* Antioxidant, Paracetamol, Hepatoprotective, Rat

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INTRODUCTION

The liver as a vital organ in the body is primarily responsible for the metabolism of carbohydrates, lipids, proteins and detoxifying xenobiotics and drugs. Thus the liver is proned to injury due to the chronic exposure to drugs, environmental toxicants and other xenobiotics [1]. The liver disorders are one of the serious health problems, throughout the world. More than 350 million people were affected with chronic hepatic infections worldwide and in India above 20,000 deaths were reported every year due to liver disorders. Hepatocellular carcinoma is one of the most common tumors in the world with over 250,000 new cases each year [2].

Acetaminophen, also known as paracetamol (PCM) is widely used to get relief from fever, headaches and pains [3]. Paracetamol is primarily metabolised by the liver and it is non toxic in sufficient doses. However, either accidental or deliberate overdose can lead to hepatotoxicity caused by the reactive metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress [4-5]. In June 2009, The Food and Drug Administration (FDA) advisory committee recommended that new restrictions should be placed to protect people from the potential toxic effects of paracetamol. So far no therapy has successfully prevented the progression of hepatic disease. Even though new drugs were developed to treat the chronic liver disorders, these drugs often have side effects [6].

In recent days, the use of herbal natural product has increased attention among the world population. Many of the herbal supplements are claimed to assist in healthy lifestyle. Medicinally, herbal drugs have made a significant contribution for the treatment of hepato toxicity [7-8]. *Allium cepa*, commonly known as garden onion is the largest and principal representative genus of the Liliaceae family. It possesses bountiful proteins, carbohydrates, sodium, potassium and phosphorus. Traditionally onion has been used to treat intestinal infections, eye infections, ear ache, urinary tract burning, headaches associated with drowsiness, ulcers on heels and cough resulted from the inspiration of cold air. Many reports revealed that onion was found to be an antibacterial, antiviral, antiparasitic, antifungal and has antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti inflammatory and antioxidant activity [9].

In recent decades, the extracts of leaves, seeds and roots of *Allium cepa* have been extensively studied for many potential uses including anti-diabetic[10], anti-tumour[11], hepatoprotective [12], nephrotoxicity [13] and antioxidant[14] activities. Keeping these folkloric claims and reports in view, the present study attempted to assess the possible hepatoprotective potential of aqueous extract of *Allium cepa* bulb in paracetamol-induced hepatotoxicity in rats.

MATERIALS AND METHOD

Plant Extract, Chemical and Drugs

The aqueous extract of *Allium cepa* bulb was supplied by M/s. Laila Impex, Vijayawada, India. 2,2-diphenyl-1-picrylhydrazil (DPPH) were purchased from Sigma Chemical Co. (St. Louis,

MO, USA). Paracetamol was obtained from Sri Krishna Pharmaceuticals, Mumbai, India. Silymarin was obtained as a gift sample from Micro Labs, Bangalore, India. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin estimated kits were procured from Span Diagnostics, Surat, India. All other chemicals and reagents used were of analytical grade.

DPPH Scavenging Assay

The DPPH scavenging activity of *Allium cepa* was measured according to the method of Liu and Zhao [15]. The reaction mixture contained 2 ml of 95% ethanol, 0.1 M DPPH and 2 ml of the *Allium cepa* (50-300 µg/ml). The solution was incubated at 25°C for 15 min, and the absorbance of *Allium cepa* was determined at 517 nm. The antioxidant activity of *Allium cepa* extract was evaluated according to the following formula:

Scavenging rate (%) = $[1-A]/ A_0 X100$

Where A was absorbance of *Allium cepa* extract and A_0 was the absorbance of DPPH solution.

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was measured according to the method of Winterbourn and Sutton[16]. The reaction mixture contained 1 ml of 0.15 M phosphate buffer saline (pH 7.4), 1 ml of 40 g/ml safranin, 1 ml of 0.945 mM EDTA-Fe (II), 1 ml of 3% (v/v) $H_2 O_2$, and 0.5 ml of the *Allium cepa* (50-300 µg/ml). After incubating at 37°C for 30 min, the absorbance of the *Allium cepa* was measured at 560 nm. The IC_{50} value of *Allium cepa* is the effective concentration at which the hydroxyl radicals were scavenged by 50%. The hydroxyl radical- scavenging activity was expressed as:

Scavenging rate (%) = $[A_0-A_1]/ A_0 X 100$

Where A_0 was absorbance of blank and A_1 was the absorbance of *Allium cepa* extract.

Superoxide Radical Scavenging Assay

Superoxide anion radical scavenging activity was determined according to the method of Stewart and Bewley[17]. The reaction mixture (3 ml) contained 13 mM methionine, 10 mM riboflavin,75 M nitroblue tetrazolium, 100 mM EDTA, 50 mM phosphate buffer (pH 7.8), and the *Allium cepa* (50-300 µg/ml). After illuminating the reaction mixture with a fluorescent lamp at 25º C for 30 min, the absorbance of the *Allium cepa* was measured at 560 nm. The scavenging rate was calculated using the following formula:

Scavenging rate (%) = $[A_0-A]/ A_0 X100$

Where A was the absorbance of *Allium cepa* and A_0 was absorbance of the blank.

Animals

Adult male albino Wistar rats (180 ± 20 g) were obtained from the Mahaveer Enterprizes, Hyderabad, India. They were kept under temperature of (23 ± 2)ºC, humidity of 50

% and 12 h:12 h of light and dark cycles, respectively. They were fed with Commercial pellet diet (Rayon's Biotechnology Pvt Ltd, India) and water was provided *ad libitum*. The prior approval for conducting the experiments in rats was obtained from our Institutional Animal Ethics Committee and our lab is approved by CPCSEA, Government of India (Regd. No. 516/01/A/ CPCSEA).

***In vivo* Hepatoprotective Study**

Preventive study

The rats were divided into six groups each group containing 6 rats.

Group 1: Control rats which received 2% gum acacia for 3 days.
Group 2: Received 2 g/kg bw of paracetamol for a period of 3 days.
Group 3: Received 2 g/kg bw of paracetamol and 100 mg/kg bw of *Allium cepa* extract simultaneously for 3 days.
Group 4: Received 2 g/kg bw of paracetamol and 300 mg/kg bw of *Allium cepa* extract simultaneously for 3 days.
Group 5: Received 2 g/kg bw of paracetamol and 600 mg/kg bw of *Allium cepa* extract simultaneously for 3 days.
Group 6: Received 2 g/kg bw of paracetamol and 100 mg/kg bw of silymarin simultaneously for 3 days.

Curative Study

Group 1: Control rats which received 2% gum acacia for 10 days.
Group 2: Received 2 g/kg bw of paracetamol for a period of 3 days.
Group 3: Received 2 g/kg bw of paracetamol daily for a period of 3 days and then received 100 mg/kg bw of *Allium cepa* extract for next 7 days.
Group 4: Received 2 g/kg bw of paracetamol daily for a period of 3 days and then received 300 mg/kg bw of *Allium cepa* extract for next 7 days.
Group 5: Received 2 g/kg bw of paracetamol daily for a period of 3 days and then received 600 mg/kg bw of *Allium cepa* extract for next 7 days.
Group 6: Received 2 g/kg bw of paracetamol for 3 days and then silymarin 100 mg/kg orally for the next 7 days**.**

Administrations were done orally. Silimarin was the reference hepatoprotective agent. In preventive study, blood samples were collected on 0^{th} and 4^{th} day and in curative study, blood samples were collected on 0^{th}, 4^{th} and 11^{th} day from rats retro-orbital plexus. Blood samples were collected into centrifuge tubes and were centrifuged at 3000 rpm for 30 min to obtain the serum, used for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin in Semi-auto analyzer (Screen master-3000).

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Data and Statistical Analysis

All analyses were performed with statistical package for social sciences (SPSS) 13.0 for Windows (SPSS, USA). The data were expressed as mean with S.D. The significance was determined by applying student's paired't' test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Effect of *Allium cepa* against the DPPH Radicals

The free radical scavenging activity of *Allium cepa* against DPPH radicals was shown in table 1. The various concentrations of *Allium cepa* and standard ascorbic acid in the dose range of 50- 600 µg/ml showed antioxidant activity in a dose dependent manner. The IC_{50} values for *Allium cepa* and ascorbic acid were found to be 195.2 and 159.7 µg/ml respectively.

Effect of *Allium cepa* against the Hydroxyl Radicals

The free radical scavenging activity of *Allium cepa* against hydroxyl radicals was shown in table 1. The various concentrations of *Allium cepa* and standard ascorbic acid in the dose range of 50- 600 µg/ml showed antioxidant activity in a dose dependent manner. The IC_{50} values for *Allium cepa* and ascorbic acid were found to be 374.7 and 244.3 µg/ml respectively.

Effect of *Allium cepa* on the Superoxide Scavenging Activity

The free radical scavenging activity of *Allium cepa* against superoxide radical was shown in table 1. The various concentrations of *Allium cepa* and standard ascorbic acid in the dose range of 50- 600 µg/ml showed antioxidant activity in a dose dependent manner. The IC_{50} values for *Allium cepa* and ascorbic acid were found to be 182.5 and 97.2 µg/ml respectively.

Table 1: DPPH, Hydroxyl, Super oxide radical scavenging activity of the *Allium cepa* extract and ascorbic acid

Conc. (µg/ml)	DPPH radical scavenging activity (% of inhibition)		Hydroxyl radical scavenging activity (% of inhibition)		Super oxide scavenging activity (% of inhibition)	
	Allium cepa	Ascorbic acid	*Allium cepa*	Ascorbic acid	*Allium cepa*	Ascorbic acid
50	25.3 ± 1.2	30.4 ± 1.4	5.6 ± 1.5	18.5 ± 1.2	21.5 ± 0.5	32.3 ± 1.2
100	36.7 ± 1.5	45.2 ± 0.4	19.4 ± 1.8	32.6 ± 0.7	39.5 ± 1.6	49.5 ± 0.6
200	51.2 ± 0.6	60.8 ± 1.9	30.6 ± 1.6	51.3 ± 1.1	60.2 ± 1.8	68.5 ± 1.2
300	62.3 ± 2.4	71.2 ± 1.6	52.5 ± 2.1	65.3 ± 0.6	78.3 ± 1.4	89.5 ± 0.3
600	70.4 ± 1.1	80.5 ± 0.9	71.3 ± 1.7	86.4 ± 0.5	92.1 ± 1.7	101.4 ± 1.3
IC_{50}	**195.2 ± 0.2**	**159.7 ± 0.7**	**374.7 ± 0.4**	**244.3 ± 0.9**	**182.5 ± 1.7**	**97.2 ± 1.4**

Values are mean ± S.D three replicates

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Estimation of Serum Biochemical Parameters

Results presented in Table 2 to 5 indicate that the levels of serum enzymes namely AST, ALT, ALP and total bilirubin levels were significantly ($p<0.01$) increased in paracetamol treated rats when compared with normal rats. However, treatments of rats with *Allium cepa* and silymarin serum enzymes like AST, ALT, ALP and total bilirubin levels were significantly ($p<0.01$) decreased when compared to paracetamol treated rats in both preventive and curative study.

Table 2: Effect of aqueous extract of *Allium cepa* bulb and silymarin on AST and ALT on paracetamol induced hepatotoxicity in wistar rats (preventive study)

Groups	AST		ALT	
	0 day	4 day	0 day	4 day
Normal rats	$20.6^{ns} \pm 2.15$	$21.4^{ns} \pm 4.2$	$20.6^{ns} \pm 4.32$	$21.6^{ns} \pm 5.72$
PCM control (2g/kg bw)	$24.5^{ns} \pm 3.61$	$68.3^{\#} \pm 5.21$	$19.5^{ns} \pm 3.15$	$68.1^{\#} \pm 4.58$
PCM (2g/kg bw) + A.C (50mg/kg bw)	$21.6^{ns} \pm 4.62$	$53.5^{*} \pm 3.05$	$20.6^{ns} \pm 4.18$	$50.8^{*} \pm 3.67$
PCM (2g/kg bw) + A.C (100 mg/kg bw)	$26.8^{ns} \pm 3.21$	$48.6^{*} \pm 2.84$	$22.5^{ns} \pm 3.84$	$43.8^{*} \pm 6.75$
PCM (2g/kg bw) + A.C (300 mg/kg bw)	$28.8^{ns} \pm 4.55$	$43.6^{*} \pm 3.45$	$24.6^{ns} \pm 5.42$	$41.3^{*} \pm 3.42$
PCM (2g/kg bw) + silymarin (100 mg/kg bw)	$28.7^{ns} \pm 5.32$	$35.6^{*} \pm 5.14$	$25.6^{ns} \pm 4.12$	$39.8^{*} \pm 5.12$

Values are expressed as Mean ± SD of 6 individuals. ns= non significant; PCM= paracetamol; A.C = *Allium cepa*
#P<0.01, compared with normal control, *P<0.01, compared with PCM control

Table 3: Effect of aqueous extract of *Allium cepa* bulb and silymarin on ALP and total bilirubin on paracetamol induced hepatotoxicity in wistar rats (preventive study)

Groups	ALP		Total bilirubin	
	0 day	4 day	0 day	4 day
Normal rats	$43.7^{ns} \pm 4.12$	$47.4^{ns} \pm 5.84$	$1.2^{ns} \pm 0.03$	$1.1^{ns} \pm 0.05$
PCM control (2g/kg bw)	$40.2^{ns} \pm 8.62$	$236.2^{\#} \pm 6.71$	$1.1^{ns} \pm 0.02$	$5.3^{\#} \pm 0.06$
PCM (2g/kg bw) + A.C (50mg/kg bw)	$46.6^{ns} \pm 3.12$	$198.9^{*} \pm 7.12$	$1.1^{ns} \pm 0.02$	$3.7^{*} \pm 0.08$
PCM (2g/kg bw) + A.C (100 mg/kg bw)	$45.8^{ns} \pm 4.15$	$178.5^{*} \pm 8.12$	$1.3^{ns} \pm 0.01$	$3.6^{*} \pm 0.04$
PCM (2g/kg bw) + A.C (300 mg/kg bw)	$44.8^{ns} \pm 6.75$	$145.6^{*} \pm 4.3$	$1.3^{ns} \pm 0.01$	$3.1^{*} \pm 0.04$
PCM (2g/kg bw) + silymarin (100 mg/kg bw)	$43.7^{ns} \pm 7.51$	$103.9^{*} \pm 6.3$	$0.8^{ns} \pm 0.02$	$1.5^{*} \pm 0.13$

Values are expressed as Mean ± SD of 6 individuals, ns= non significant; PCM= paracetamol; A.C = *Allium cepa*
#P<0.01, compared with normal control,*P<0.01, compared with PCM control

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Table 4: Effect of aqueous extract of *Allium cepa* bulb and silymarin on AST and ALT on paracetamol induced hepatotoxicity in wistar rats (curative study)

Groups	AST			ALT		
	0 day	4 day	11 day	0 day	4 day	11 day
Normal rats	27.5^{ns} ± 4.1	28.6^{ns} ± 3.2	30.7^{ns} ± 4.01	20.1^{ns} ± 3.5	22.6^{ns} ± 4.2	21.8^{ns} ± 3.7
PCM control (2g/kg bw)	34.2^{ns} ± 4.7	185.4$^{\#}$ ± 6.1	192.5$^{\#}$ ± 2.85	22.8^{ns} ± 3.8	105.6$^{\#}$ ± 8.7	101.3$^{\#}$ ± 6.8
PCM (2g/kg bw) + A.C (50mg/kg bw)	30.8^{ns} ± 5.5	168.5* ± 7.3	142.8* ± 4.76	24.6^{ns} ± 4.3	80.7* ± 5.9	68.2* ± 8.5
PCM (2g/kg bw) + A.C (100 mg/kg bw)	27.4^{ns} ± 5.4	185.7* ± 5.8	124.9* ± 5.84	19.7^{ns} ± 4.8	75.6* ± 4.3	49.5* ± 4.3
PCM (2g/kg bw) + A.C (300 mg/kg bw)	29.7^{ns} ± 3.3	150.9* ± 8.2	103.2* ± 7.82	23.6^{ns} ± 3.6	73.3* ± 6.4	43.7* ± 3.2
PCM (2g/kg bw) + silymarin (100 mg/kg bw)	32.4^{ns} ± 3.6	131.3* ± 9.2	40.9* ± 5.82	26.5^{ns} ± 2.4	62.5* ± 6.42	36.6* ± 4.3

Values are expressed as Mean ± SD of 6 individuals,ns= non significant; PCM= paracetamol; A.C = *Allium cepa,* #P<0.01, compared with normal control, *P<0.01, compared with PCM control

Table 5: Effect of aqueous extract of *Allium cepa* bulb and silymarin on ALP and total bilirubin on paracetamol induced hepatotoxicity in wistar rats (curative study)

Groups	ALP			Total bilirubin		
	0 day	4 day	11 day	0 day	4 day	11 day
Normal rats	35.3^{ns} ± 5.8	38.2^{ns} ± 4.2	40.2^{ns} ± 4.2	0.2^{ns} ± 0.04	0.2^{ns} ± 0.02	0.2^{ns} ± 0.06
PCM control (2g/kg bw)	45.6^{ns} ± 4.2	295.2$^{\#}$ ± 16.5	275.8$^{\#}$ ± 14.5	0.5^{ns} ± 0.03	4.6$^{\#}$ ± 0.05	4.3$^{\#}$ ± 0.02
PCM (2g/kg bw) + A.C (50mg/kg bw)	40.8^{ns} ± 6.7	258.6* ± 18.5	163.5* ± 8.03	0.3^{ns} ± 0.01	3.9* ± 0.02	2.9* ± 0.04
PCM (2g/kg bw) + A.C (100 mg/kg bw)	33.4^{ns} ± 7.8	232.8* ± 15.6	93.8* ± 6.9	0.3^{ns} ± 0.03	3.6* ± 0.08	2.4* ± 0.04
PCM (2g/kg bw) + A.C (300 mg/kg bw)	37.6^{ns} ± 4.9	212.7* ± 18.2	80.4* ± 9.4	0.5^{ns} ± 0.02	3.4* ± 0.06	2.1* ± 0.03
PCM (2g/kg bw) + silymarin (100 mg/kg bw)	44.5^{ns} ± 4.5	190.6* ± 20.6	65.3* ± 8.6	0.5^{ns} ± 0.05	2.9* ± 0.21	2.7* ± 0.04

Values are expressed as Mean ± SD of 6 individuals, ns= non significant; PCM= paracetamol; A.C = *Allium cepa*, #P<0.01, compared with normal control, *P<0.01, compared with PCM control

DISCUSSION

Paracetamol is a well known antipyretic and analgesic agent. In excess doses, it causes severe hepatic damage in humans and experimental animals. It is used commonly as a model for hepatotoxicity. Formation of a reactive metabolite viz. N-acetyl-p-benzoquinoneimine (NAPQI) by the action of cytochrome P-450 enzyme system contributes to the hepatotoxicity of

the paracetamol [18]. This generates the reactive oxygen species, thereby enhances oxidative stress which leads to liver injury and hepatocellular death [19-20]. When there is damage to the liver cell membrane, the cytosolic enzymes are leaked into the blood stream [21]. Therefore, the elevation of these cytosolic enzymes in the blood stream is a needful quantitative marker of the extent of hepatic damage. The elevated levels of the AST, ALT, ALP and total bilirubin levels in the rats administered with paracetamol indicate the hepatocellular damage and alterations in the membrane permeability. Our reports on these elevated levels during paracetamol induced hepatic damage are in accordance with the previous reports [19]. Pre-treatment and after-treatment with the *Allium cepa* attenuated the elevated levels of ASP, ALT, ALP and total bilirubin levels.

Earlier studies demonstrated that bulb of *Allium cepa* had reduced elevated AST, ALT and ALP levels in rodents [12], and also another study has showed that leaf extracts of *Allium cepa* had significantly restored the elevated AST, ALT and ALP enzyme levels to the normal levels[22]. Recently Riyaz Shaik et al. [23] demonstrated that *Allium cepa* leaves protect the hepatocytes by preventing the release of these 3 enzymes. Our results are consistent with earlier studies, which strongly suggest that *Allium cepa* may protect the structural integrity of hepatocytes and prevent the release of cytosolic enzymes into bloodstream.

Additionally, *Allium cepa* showed effective DPPH, hydroxyl, super oxide radical scavenging activity, suggesting that it could scavenge the free radicals generated during paracetamol metabolism. This finding is consistent with previous studies which demonstrated the antioxidant activity of *Allium cepa* extract [24]. The antioxidant property of *Allium cepa* may be due to bioactive compounds flavonoids, phenolic compound, pectic polysaccharide [25-26]. These compounds quench ROS and regenerate membrane-bound antioxidants levels during aadministration of *Allium cepa* at different dose levels in preventive and curative studies.

CONCLUSIONS

The present study concludes that the *Allium cepa* bulb aqueous extract possesses antioxidant activity and shows a protective effect against paracetamol induced hepatotoxicity in experimental rats. However, further investigation is in process on the seeds extract to identify the active constituents' responsibility for hepatoprotection.

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