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Original Article

AMELIORATIVE EFFECTS OF STEM BARK OF THE WONDER TREE, *PROSOPIS CINERARIA* (L.) DRUCE AGAINST LPS-INDUCED TOXICITY: AN *IN VITRO* STUDY

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ABSTRACT

Objective: The present experimental investigation was planned to unravel and analyze the therapeutic potential of hydro-ethanol extract prepared from the stem bark of *Prosopis cineraria* against LPS-induced toxicity under *in vitro* conditions.

Methods: Liver tissue samples from healthy Swiss albino male mice (*Mus musculus*) were used for the study. Liver homogenate (0.9 ml) was treated with 0.05 mg/ml of LPS along with 0.01 to 0.05 mg/ml of hydro-ethanol plant extract and allowed to incubate at 37˚C. The reactions were terminated at different time points at 0 min, 30 min, 1 h, 2 h, 4 h, 8 h and 24 h and alterations in oxidative stress (LPO, CAT, SOD, GSH, GST, and GPx) and biochemical parameters of hepatic toxicity (AST and ALT, ACP and ALP) were studied.

Results: The results demonstrated that the obliterations in the levels of oxidative and biochemical parameters due to LPS induced toxicity were restored by the treatment with hydro-ethanol extract of *Prosopis cineraria* under *in vitro* conditions. The altered levels were biochemical parameters were observed at 0.05 mg/ml LPS concentration after 2 h; but administration of hydro-ethanol plant extract at concentration 0.04 mg/ml effectively reduced its level when compared to LPS treated samples under *in vitro* conditions

Conclusion: The present research work unravelled the alleviating potential of a hydro-ethanol extract of *Prosopis cineraria* against LPS-induced toxicity by combating oxidative stress under *in vitro* environment.

Keywords: Antioxidants, *In vitro*, *Prosopis cineraria*, Reactive oxygen species, Liver homogenate

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INTRODUCTION

Lipopolysaccharides (LPS), also referred to as endotoxins, found in the outer monolayer of most gram-negative bacteria, is known for triggering an innate immune response and inflammatory cascade in the host. The excessive and prolonged inflammatory response triggered by LPS in the host can lead to vascular leakage, septic shock or endotoxin shock, tissue and organ damage and can eventually cause death [1]. LPS has potentially attracted the curiosity of researchers due to its pathogenic role in numerous human and animal diseases [2]. The intensity of the response elicited by LPS depends on its dose and varies from species to species. It is reported that higher animals, humans are more sensitive even at low doses of LPS on the contrary, lower animals are quite resistant to LPS-induced deleterious effects [3]. Studies indicate the massive generation of reactive oxygen species (ROS) in LPS-induced signalling cascade [4]. ROS are known to have biocidal effects on invading micro-organisms and are important components of innate immune response. ROS includes singlet oxygen, superoxide anion radical, highly reactive hydroxyl radical and hydrogen peroxide. However, generation of ROS can potentially harm the tissues and organs of the host [5, 6]. The lethal effects of LPS are known to be elicited through activation of macrophages, neutrophils which consequently mediate the release of pro-inflammatory cytokines and other bioactive inflammatory mediators, including Tumour Necrosis Factor α (TNF-α), Interleukin-6 (IL-6) and Nitric oxide (NO). LPS exerts stimulation of antibody production, B-cell proliferation, and activation of T-lymphocytes to produce cytokines [2].

Most common therapeutic treatment for bacterial infections is the administration of antibiotics. But ironically, the use of antibiotics for curing gram-negative bacterial infections can increase endotoxin load and exaggerated inflammatory response. This is due to the fact that antibiotics kill the bacteria but the cell components mainly LPS, continue to elicit an inflammatory response in the host [7]. Thus, in order to develop therapy or effective new drugs for sepsis, the detailed understanding of triggering of inflammatory response and mechanisms underlying the clinical manifestation of serious diseases in the host is indispensable.

India has a tremendous wealth of medicinal plants which are the store-house of various bioactive entities that possess therapeutic properties against numerous diseases. Since ancient times, the importance of plants as a source of medicine has been realized and many medicinal plants are being used as folk medicines for the amelioration of various serious diseases. *Prosopis cineraria* (L.) Druce, commonly known as 'Khejri', is the state tree of Rajasthan [8]. *Prosopis cineraria* has therapeutic importance and it is used for curing serious diseases and possess pharmacological activities like anti-fungal, anthelmintic, anti-cancer, anti-bacterial, anti-viral, anti-hyperglycemic, anti-hyperlipidemic, anti-oxidative [9]. The bark of *P. cineraria* is used in the treatment of various ailments such as asthma, leprosy, wandering of mind, fever, dyspepsia, dysentery, rheumatism, muscle tremors, bronchitis, piles, leucoderma [10, 11]. Numerous Phyto-constituents like tannins, steroids, flavone derivatives (namely Prosogerin A, B, C and E), Rutin, Patulitrin, Luteolin, Patuletin, alkaloids etc. have been reported and isolated from different parts of this medicinal plant [12].

In vitro studies are advantageous as they not only provide indication about the possible *in vivo* effects of the toxicant or the plant samples under investigation but also reduce time and efforts of the researchers. It must be acknowledged that *in vitro* studies also help in minimizing the number of animals needed to be sacrificed for the research work. The therapeutic potential of *Prosopis cineraria* has been extensively explored by many eminent researchers against various serious diseases but its activity against Lipopolysaccharide (endotoxin) is obscure. Therefore, the present research study was executed to elucidate the ameliorative effects of stem bark of *Prosopis cineraria* against LPS-induced toxicity under *in vitro* conditions so, that the implication about the possible potential of the plant extract can be deduced with minimal animal sacrifice.

MATERIALS AND METHODS

Chemicals

Lipopolysaccharide (from *Escherichia coli* 0111:B4) was procured from Sigma-aldrich. The chemicals and all the reagents mentioned in the present experimental research were of high purity (98-99%) and analytical grade. Chemicals were purchased from reliable firms and sources.

Experimental plant

The stem-bark of *Prosopis cineraria* was procured from the local region of Banasthali Vidyapith, Rajasthan. The collected plant was identified by Farm Manager at Krishi Vigyan Kendra, Banasthali Vidyapith, Rajasthan and authenticated at Herbarium unit of Banasthali Vidyapith, Rajasthan, India (Herbarium No.-BVRI1359/2017).

Preparation of plant extract

The plant material i.e. stem bark was shade dried and then powdered by using mixer grinder. The powdered stem-bark (30 g) was packed in a thimble and subjected to soxhlet extraction. The powdered material of the experimental plant was extracted with Pet ether and then the obtained marc was subjected to soxhlet extraction by 80% ethanol.

The extract was evaporated to dryness using rotary evaporator and stored in airtight jars at 4 ° C for further experimental usage. For experimental use, mixed the plant extracts with the respective solvent to achieve a concentration of 1 mg/ml.

Experimental animals

The experimental animal model *i.e.* male Swiss Albino mice (*Mus musculus*) weighing between 15-30 g were procured from Lala Lajpat Rai University, Hissar (India). (Ref. No. BU/BT/402/14-15).

Maintenance of animal models

Swiss Albino adult male mice (*Mus musculus*) were maintained in a duly-ventilated animal house with 12 h light-dark cycle. Swiss albino mice were housed (six mice per cage) in polypropylene cages in an air-conditioned room with ambient temperature (25±3˚C), humidity (50±15%). Proper hygienic and sterile conditions were maintained in the animal house facility. The mice were fed with a healthy pelleted diet [Hindustan lever limited, India: metal content in parts per million (ppm) dry weight: Cu-10.0, Zn-45.0, Mn-55.0, Co-5.0 and Fe-75.0] and drinking water *ad libitum* throughout the experimental study.

Liver sample

Fresh liver samples of healthy Swiss albino male mice weighing approximately 1.6 gm were dissected out after cervical dislocation of the mice.

Preparation of liver homogenate

1.6 gm of liver was homogenized in 16 ml of phosphate buffer solution then centrifuged (at 9000 rpm) for the removal of cell debris for the estimation of various biochemical parameters. During the preparation of liver homogenate temperature was maintained at 4˚C.

LPS solution

Stock solution of LPS (1 mg/ml) was prepared by dissolving 1 mg of LPS in 1 ml of double-distilled water.

Experimental regime

To determine the ameliorative effect of medicinal plant *Prosopis cineraria* on LPS-induced changes in liver homogenate.

1. Control tubes: Tubes contained 0.9 ml of liver homogenate and 0.1 ml of distilled water.

2. LPS treated tubes: 0.05 ml of LPS was mixed with 0.9 ml of homogenate. The final volume was made upto 1 ml with distilled water.

3. Plant extract treated tubes: 0.05 ml of plant extract was mixed with 0.9 ml of liver homogenate. The final volume was made up to 1 ml with distilled water.

4. LPS and Plant extract-treated tubes: 0.05 ml of LPS solution and 0.01 ml to 0.05 ml of plant extract were mixed with 0.9 ml of liver homogenate. The final volume was made up to 1 ml with distilled water.

All the tubes were subjected to incubation at 37˚C and the parameters are analyzed at different time period-0 min, 30 min, 1 h, 2 h, 4 h, 8 h and 24 h.

Estimation of various oxidative stress and biochemical parameters

Lipid peroxidation, (LPO) (Nwanjo and Ojiako, 2005) [13]; Superoxide dismutase (SOD) (Marklund and Marklund, 1974) [14];Catalase (CAT) (Aebi,1984) [15]; Reduced glutathione (GSH) (Ellman, 1959) [16]; Glutathione S-transferase (GST) (Habig *et al.*, 1974) [17]; Glutathione peroxidase (GPx) (Rotruck *et al.*, 1973) [18]; Protein estimation (Lowry *et al.*, 1952) [19]; Aspartate transaminase (AST) and Alanine transaminase (Reitman *et al.*, 1957) [20]; Alkaline phosphatase (ALP) and Acid phosphatase (ACP) (Sadashivam, 1996) [21].

Statistical analysis

The results of the experiments were interpreted as a mean±standard deviation of triplicates. The experimental data obtained was analyzed by one way ANOVA (analysis of variance) followed by student's t-test and tukey range test using the SPSS 16.0 (Statistical Program for Social Sciences) program. The experimental results with $p<0.05$ were considered as satisfactory and $p<0.001$ were considered as highly significant.

RESULTS

All the results of the experimental investigation of oxidative stress and biochemical parameters are tabulated in table 1. Various oxidative stress markers (i.e. LPO, SOD, CAT, GSH, GST and GPx) and biochemical markers (AST and ALT, ALP and ACP) have been studied. Total protein content (TPC) was also estimated to compare the extent of alteration and for the calculation of enzymes specificity with respect to protein content (table 1 (a)). Administration of different concentrations of LPS into liver homogenate of mice led to rise in lipid peroxidation level (LPO) in comparison to control samples (table 1(b)). Our results demonstrated that the addition of 0.05 mg/ml LPS showed significant alteration in LPO, protein content, SOD, CAT, GSH, GST and GPx. The administration of hydro-ethanol plant extract at concentration 0.04 mg/ml effectively reduced its level when compared to LPS treated samples under *in vitro* conditions. Results also showed decreased SOD, CAT and TPC in LPS treated samples in comparison to control tissue samples. SOD, CAT and TPC were positively affected by the treatment of *P. cineraria*. SOD activity was increased in plant treated tissue samples in comparison to LPS intoxicated samples (table 1(c)). Plant extract at 0.04 mg/ml has capability to restore CAT activity (table 1(d)). LPS intoxicated liver homogenate samples also showed decrease in GSH content, GST and GPx activities in comparison to control samples of liver tissue. Plant treated liver homogenate samples showed increased GSH content (table 1 (e)), GST activity (table 1 (f)) and GPx (table 1(g)) in comparison to LPS treated liver homogenate. The results highlighted the ameliorative role of hydro-ethanol plant extract of *Prosopis cineraria* against LPS induced hepatic toxicity by measuring several hepato-toxic markers i.e. AST (Aspartate amino transferase) (table 1 (h)), ALT (Alanine aminotransferase) (table 1 (i)), ACP (Acid Phosphatase) (table (j)) and ALP (Alkaline Phosphatase) (table (k)). The study showed that after 2 h of incubation the levels of biochemical and oxidative stress parameters were altered noticeably in samples analyzed after 2 h and 4 h, in comparison to 0 min, 30 min and 1 h samples. However the samples retrieved after 8 h and 24 h, the levels of the studied parameters showed marginal difference. The ameliorative effect of bark extract was prominent in the samples retrieved after 2 h incubation.

Table 1: Representation of Protein (a), LPO (b) SOD (c), CAT (d), GSH (e), GPx (f), GST (g), AST (h), ALT (i), ACP (j) and ALP (k) levels in liver homogenate treated with different concentrations of plant extract and LPS

(a)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*Total protein(mg/g)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	13.14±0.07	13.14±0.08	13.11±0.79	13.10±0.10	13.04±0.71	12.74±0.12	12.14±0.73
LPS Treated	0.05	-	13.13±0.19	13.03±0.13	12.43±0.50	11.64±0.12	10.03±0.10[a]	09.63±0.14	09.43±0.19*
Plant Treated	-	0.05	13.12±0.20	13.01±0.10*	13.23±0.12	13.04±0.43	13.06±0.16	12.93±0.18	12.83±0.29
LPS+Plant treated	0.05	0.01	13.13±0.11	13.06±0.47	12.13±0.53	11.09±0.14*	10.73±0.12	09.65±0.13	09.13±0.21
		0.02	13.13±0.29	13.03±0.18	12.91±0.64	11.68±0.11*	10.93±0.17	10.63±0.27	09.93±0.26
		0.03	13.13±0.22	13.02±0.10	12.43±0.55	11.64±0.10	10.53±0.19[a]	09.61±0.49	09.54±0.21
		0.04	13.13±0.14	13.01±0.29	12.92±0.36	11.94±0.13	11.13±0.24	11.02±0.79[a]	10.43±0.24
		0.05	13.13±0.25	13.03±0.21	12.13±0.51*	11.60±0.18	10.43±0.21	10.13±0.23	10.07±0.29*

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group.

(b)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*LPO (nmolesof MDA formed/mg tissue)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	12.14±0.77	12.16±0.08	12.22±0.12	12.24±0.10	12.24±0.71	12.19±0.12	12.17±0.13
LPS Treated	0.05	-	12.16±0.13	12.12±0.24	18.75±0.26*	21.52±0.16	23.12±0.37	23.70±0.88[a]	23.81±0.19
Plant Treated	-	0.05	12.19±0.14	12.32±0.35	12.72±0.17	12.50±0.10	13.22±0.18	13.71±0.11	13.12±0.20*
LPS+Plant treated	0.05	0.01	12.15±0.15	12.92±0.14	18.72±0.18	21.22±0.11	23.42±0.19*	23.12±0.09	23.82±0.24
		0.02	12.12±0.16	12.92±0.27	18.72±0.49	21.52±0.19	23.52±0.10	23.72±0.21*	23.82±0.02
		0.03	12.15±0.37	12.92±0.18	18.72±0.20	21.52±0.25	23.52±0.21[a]	23.72±0.27	23.82±0.23
		0.04	12.19±0.18	12.95±0.11	19.12±0.20	19.10±0.01*	17.45±0.22	17.19±0.13	16.09±0.24*
		0.05	12.14±0.11	12.65±0.21	18.15±0.23	17.35±0.12	16.18±0.20	16.11±0.14*	15.15±0.25

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. **(LPO**-Lipid peroxidation)

(c)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*SOD(U/min/mg protein)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	0.05±0.70	0.05±0.08	0.04±0.78	0.04±0.12	0.04±0.18	0.04±0.05	0.04±0.98
LPS Treated	0.05	-	0.05±0.73	0.04±0.11	0.03±0.13	0.03±0.16*	0.02±0.11	0.02±0.16	0.02±0.13*
Plant Treated	-	0.05	0.05±0.76	0.05±0.08	0.04±0.78	0.04±0.12	0.04±0.18	0.03±0.05*	0.03±0.98
LPS+Plant treated	0.05	0.01	0.05±0.79	0.04±0.12	0.03±0.13	0.03±0.16	0.02±0.11*	0.02±0.16	0.02±0.23
		0.02	0.05±0.82	0.04±0.08	0.04±0.78	0.03±0.12	0.03±0.18	0.02±0.05	0.02±0.98*
		0.03	0.05±0.85	0.04±0.13	0.03±0.13	0.03±0.16	0.02±0.11[a]	0.02±0.16	0.02±0.13
		0.04	0.05±0.88	0.05±0.08	0.05±0.78	0.04±0.12*	0.04±0.18	0.04±0.05	0.03±0.98*
		0.05	0.05±0.91	0.05±0.14	0.05±0.13	0.04±0.16*	0.04±0.11	0.04±0.16	0.04±0.13

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. **(SOD**-Superoxide dismutase)

(d)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*CAT(µmole of H202 consumed/min/mg protein)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	155.94±0.74	155.84±0.15	153.12±0.86	152.14±0.71	151.14±0.32	151.03±0.19	150.14±0.80
LPS Treated	0.05	-	155.14±0.17	151.98±0.36	142.20±0.65	139.72±0.16*	136.72±0.77*	134.72±0.38	134.12±0.10
Plant Treated	-	0.05	154.19±0.40	154.11±0.10	152.34±0.16	152.04±0.11	152.24±0.39	151.11±0.11	151.04±0.86
LPS+Plant treated	0.05	0.01	152.10±0.97	151.91±0.37	142.21±0.66	139.82±0.17*	136.12±0.78	134.92±0.39	134.02±0.11
		0.02	152.11±0.15	152.04±0.15	149.19±0.86	139.04±0.71	138.18±0.32	137.14±0.19	132.04±0.80*
		0.03	152.14±0.21	151.98±0.38	142.20±0.67	139.72±0.18	136.72±0.79	134.72±0.40	134.12±0.12
		0.04	155.94±0.26	151.10±0.15	148.14±0.46	152.19±0.01*	153.14±0.32[a]	152.14±0.19	152.84±0.80
		0.05	155.74±0.32	151.98±0.39	142.20±0.68	150.72±0.19[a]	151.72±0.80*	154.72±0.41	154.12±0.13

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. **(CAT-**Catalase)

(e)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*GSH(µmole GSH/g tissue)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	3.16±0.12	3.16±0.93	3.15±0.74	3.14±0.25	3.14±0.96	3.13±0.37	3.13±0.78
LPS Treated	0.05	-	3.15±0.75	3.08±0.44	3.03±0.95	3.00±0.06*	2.98±0.17	2.96±0.18	2.95±0.19
Plant Treated	-	0.05	3.15±0.138	3.15±0.93	3.14±0.74	3.14±0.25	3.14±0.96	3.14±0.37	3.13±0.78
LPS+Plant treated	0.05	0.01	3.15±0.01	3.09±0.45	3.02±0.96	3.02±0.07	2.99±0.18*	2.92±0.19	2.91±0.20
		0.02	3.17±0.24	3.15±0.91	3.04±0.71	3.04±0.15	2.94±0.16	3.04±0.31	3.01±0.82
		0.03	3.15±0.37	3.08±0.46	3.03±0.97	3.00±0.08	2.98±0.19*	2.96±0.20	2.95±0.21
		0.04	3.18±0.390	3.15±0.93	3.15±0.74	3.14±0.25*	3.14±0.96	3.14±0.37	3.14±0.78
		0.05	3.16±0.453	3.08±0.47	3.03±0.98	3.00±0.09	3.08±0.20	3.06±0.21*	3.15±0.22

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. **(GSH**-Reduced glutathione)

(f)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*GST(nmole CDNB-GSH conjugate formed/min/mg protein)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	525.04±0.74	525.54±0.15	523.01±0.70	523.11±0.17	521.29±0.11	520.11±0.79	520.04±0.10
LPS Treated	0.05	-	524.94±0.77	511.17±0.08	500.14±0.19	495.14±0.10*	493.14±0.81	490.14±0.02	485.14±0.13
Plant Treated	-	0.05	526.14±0.80	525.85±0.75	525.13±0.26	524.24±0.47	524.94±0.78	523.19±0.29	523.23±0.80
LPS+Plant treated	0.05	0.01	525.10±0.83	521.14±0.79	520.53±0.30	497.14±0.40	495.14±0.81	496.11±0.32*	498.89±0.83
		0.02	524.21±0.86	520.13±0.75	520.17±0.39*	520.34±0.73	520.04±0.38	520.04±0.19	520.45±0.34
		0.03	525.12±0.89	515.12±0.10	517.18±0.71	515.11±0.07*	518.24±0.81	521.14±0.12	525.31±0.83
		0.04	526.15±0.92	525.14±0.15	525.16±0.76	524.14±0.43*	523.14±0.78	523.04±0.19[a]	520.13±0.80
		0.05	524.17±0.95	521.10±0.81	520.12±0.84	525.19±0.60*	525.54±0.21	529.21±0.12	531.04±0.83*

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. **(GST**-Glutathione-s-transferase)

(g)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*GPx(nM NADH oxidised/mg protein)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	26.24±0.37	26.04±0.08	26.10±0.39	25.09±0.40	25.06±0.51	25.14±0.92	24.04±0.13
LPS Treated	0.05	-	25.10±0.40	23.64±0.48	20.44±0.34	19.17±0.70	19.19±0.47	18.14±0.12	17.91±0.03*
Plant Treated	-	0.05	25.24±0.38	25.14±0.09	25.10±0.40	24.09±0.11	24.06±0.52	24.14±0.93	24.94±0.14
LPS+Plant treated	0.05	0.01	25.17±0.41	23.11±0.49	20.14±0.31	19.18±0.70	19.34±0.41	19.04±0.12[a]	19.19±0.93
		0.02	24.14±0.39	24.14±0.10	23.10±0.41	22.09±0.12*	21.06±0.53	20.14±0.94[a]	20.94±0.15
		0.03	25.10±0.82	24.94±0.50	24.45±0.32	23.11±0.43	22.14±0.90*	20.18±0.12	19.34±0.43
		0.04	25.24±0.40	24.04±0.61	24.04±0.45	24.21±0.13*	23.29±0.44	22.19±0.40	20.18±0.16
		0.05	25.04±0.38	24.11±0.09	24.10±0.40[a]	24.09±0.11	23.06±0.52	23.14±0.93	20.94±0.14

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. **(GPx-**Glutathione peroxidase)

(h)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*AST(µmole Pyruvate formed/min/mg protein)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	195.55±0.77	192.14±0.08	192.90±0.19	190.24±0.40	188.64±0.81	186.51±0.22	182.03±0.13
LPS Treated	0.05	-	196.50±0.80	200.28±0.44	219.30±0.40	224.07±0.18*	228.51±0.39	233.54±0.23	237.17±0.11
Plant Treated	-	0.05	198.14±0.63	196.07±0.08	196.21±0.71	199.54±0.89	198.59±0.01	192.14±0.22	192.04±0.13
LPS+Plant treated	0.05	0.01	197.24±0.16	198.25±0.45	217.38±0.41	220.07±0.18	221.51±0.16	230.56±0.20	232.37±0.29*
		0.02	197.50±0.09	192.14±0.78	212.59±0.09	210.04±0.89[a]	208.24±0.81*	223.01±0.12	220.59±0.03
		0.03	195.59±0.02	194.28±0.46	210.30±0.42*	200.57±0.18	198.07±0.19	196.17±0.20	195.52±0.21
		0.04	195.14±0.15	194.54±0.06	201.94±0.97	192.53±0.78	191.04±0.09	188.84±0.17	182.02±0.10
		0.05	196.74±0.28	198.28±0.47	195.30±0.43	199.59±0.18	200.53±0.09	187.97±0.20	181.07±0.01

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. (**AST-**aspartate transferase).

(i)

Group	Treatment LPS (mg/ml)	Plant extract (mg/ml)	*ALT(µmole Pyruvate formed/min/mg protein)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	343.40±0.77	354.14±0.08	361.04±0.72	369.14±0.80	367.46±0.11	364.04±0.02	361.67±0.89
LPS Treated	0.05	-	347.94±0.80	350.57±0.31	377.30±0.40	380.57±0.18	386.58±0.11	388.07±0.20[a]	390.51±0.11
Plant Treated	-	0.05	347.18±0.83	357.14±0.68	363.14±0.39	367.12±0.80	365.10±0.11	367.84±0.82	360.10±0.53
LPS+Plant treated	0.05	0.01	350.21±0.06	352.50±0.32	372.30±0.41	377.87±0.08	380.57±0.19	381.97±0.90	382.56±0.21
		0.02	347.17±0.89	357.94±0.78	371.04±0.09	377.19±0.80	377.14±0.89	371.84±0.12	377.15±0.93
		0.03	348.14±0.02	360.58±0.33	377.30±0.42	380.67±0.18*	380.12±0.89	383.57±0.20	387.57±0.01
		0.04	348.11±0.95	367.04±0.06	371.84±0.17*	377.14±0.08	369.18±0.19	360.94±0.10	367.19±0.11
		0.05	347.17±0.08	367.14±0.99	371.04±0.10	375.18±0.01	361.14±0.72	369.34±0.17	366.94±0.14

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. (**ALT**-alanine transferase).

(j)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*ACP(µmole pNP released/min/mg protein)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	5.14±0.17	5.12±0.11	5.06±0.19	4.90±0.23	4.99±0.21	4.95±0.22	4.90±0.13
LPS Treated	0.05	-	4.94±0.20	4.09±0.14	4.01±0.95*	3.97±0.04	3.82±0.62	3.77±0.18	3.67±0.19
Plant Treated	-	0.05	4.94±0.11	4.09±0.15	4.01±0.16	4.74±0.66	4.70±0.67	4.50±0.68	4.17±0.60
LPS+Plant treated	0.05	0.01	4.19±0.22	4.19±0.36	4.11±0.97	3.99±0.16	3.96±0.58	3.97±0.62	3.95±0.19
		0.02	4.17±0.23	4.39±0.17	4.41±0.18	4.07±0.66*	4.06±0.67	4.17±0.68	3.94±0.19
		0.03	4.94±0.04	4.45±0.18	4.54±0.99	4.16±0.09*	4.01±0.14	3.99±0.08[a]	3.97±0.63
		0.04	4.55±0.15	4.61±0.26[a]	4.74±0.17	4.82±0.28*	4.14±0.09	4.10±0.30	4.11±0.11
		0.05	4.84±0.20	4.74±0.21	4.72±0.29	4.80±0.85	4.24±0.83	4.17±0.30[a]	4.19±0.39

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. (**ACP–**Acid phosphatise).

(k)

Group	Treatment LPS(mg/ml)	Plant extract (mg/ml)	*ALP(µmole pNP released/min/mg protein)*						
			at 0 min	at 30 min	at 1 h	at 2 h	at 4 h	at 8 h	at 24 h
Control	-	-	1.14±0.07	1.14±0.78	1.13±0.09	1.13±0.70	1.13±0.11	1.12±0.02	1.12±0.13
LPS Treated	0.05	-	1.14±0.10	1.18±0.64	1.23±0.35	1.57±0.36*	1.59±0.16	1.65±0.58[a]	1.77±0.19
Plant Treated	-	0.05	1.14±0.73	1.14±0.25	1.14±0.36	1.13±0.86	1.15±0.17	1.16±0.10	1.18±0.12*
LPS+Plant treated	0.05	0.01	1.13±0.16	1.17±0.06	1.22±0.37	1.56±0.12	1.54±0.17	1.62±0.48	1.72±0.69
		0.02	1.14±0.19	1.14±0.47	1.21±0.08	1.54±0.06	1.52±0.17*	1.59±0.15	1.54±0.11
		0.03	1.14±0.22	1.12±0.48	1.19±0.39	1.39±0.66	1.47±0.17	1.41±0.38[a]	1.37±0.09
		0.04	1.15±0.25	1.13±0.06	1.14±0.77	1.24±0.28*	1.27±0.19	1.28±0.10	1.24±0.71
		0.05	1.14±0.28	1.12±0.72	1.14±0.31	1.19±0.11[a]	1.24±0.02	1.29±0.33	1.20±0.74

Values are represented as mean±SD (n=6).*p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. (**ALP-**Alkaline Phosphatase).

DISCUSSION

The present investigative study was conducted to determine toxic effects of LPS under *in vitro* environment by studying various biochemical parameters which indicate oxidative stress, tissue damage or organ failure.

Our results showed that addition of 0.05 mg/ml LPS showed significant alteration in LPO, protein content, SOD, CAT, GSH, GST and GPx. The alteration in the levels of biochemical parameters may be because of liberation of ROS due to LPS-induced toxicity. LPS intoxication leads to marked increase in lipid peroxidation (table 1 (b)), which is considered as an important action in triggering the manifestation of various serious disorders. Lipid peroxidation is a process which occurs in the presence of some reactive oxygen species and it is a chain-reaction which is self-propagating, the initial oxidation of few lipid molecules can cause tissue damage [22]. The present study also showed the generation of ROS which further causes cellular toxicity. Lipid peroxidation consequently leads to the breakdown of lipids and to the formation of primary oxidation and secondary products including MDA, which can further react with thiol and amino groups, the aldehydes are known to have more diffusing ability than free radicals, which signifies that the damage can be extended to even distant sites. SODs carry out defensive action against reactive oxygen species (ROS)-triggered injury [23]. The experimental findings showed that there is a decrease in SOD levels in the LPS-treated group (table 1(c)). SOD may serve as an inhibitory agent of neutrophil-mediated inflammation and may stand for a novel restorative response for the ROS-dependent tissue injury induced by neutrophils through several mechanisms [24]. Extracellular Superoxide Dimutase, Mn-SOD and Cu, Zn-SOD have been considered as potent inhibitor of inflammatory cascade by researchers [25-27].

The present experimental study was planned to analyse the activity of catalase against LPS-induced toxicity (table 1 (d)). Catalase is the major antioxidant enzyme which play key role in conversion of H_2O_2 into water and O_2. Altered catalase activity renders disrupted removal of reactive oxygen species [28]. Over expression of catalase enzyme has been demonstrated to alleviate ROS-induced cell damage while lower levels of catalase promote autophagic cell death [29, 30]. Glutathione, GST and GPx (Glutathione peroxidase) play remarkable role in protection mechanisms against hepato-toxicity. There was lower expression of GST in LPS-treated group (table 1(f)). Glutathione Peroxidase (GPx) (table 1 (g)) helps in detoxification of peroxides with GSH by acting as an electron donor. During oxidative stress, GPx converts GSH into GSSG whereas another enzyme GR (Glutathione reductase) causes reversible conversion of GSH [31]. Previously published report suggests that the LPS intoxication leads to severe oxidative stress and significant depletion in the levels of GSH shown in table 1 (e) [32]. Since reactive oxygen species mediated oxidative imbalance have been clearly studied that could be responsible for hepatic toxicity induced by LPS, hence, ALT activity (table 1(i)) was much higher than the AST activity (table 1(h)) in hepato-toxic conditions as observed after LPS intoxication in mice. ALP (Alkaline phosphatase) and ACP (Acid phosphatase) (table 1(j)) both have tremendous importance in diagnosis of hepatic deterioration. ALP occurs in sinusoidal and bile canaliculi membranes of liver and is also associated with numerous biological activities (like protein synthesis, metabolic transport across cell membrane, secretary activity and glycogen metabolism) [33, 34]. The alteration in ALP activity (table 1 (k)) is linked with the damage in membrane permeability that leads to cell damage.

Medicinal plants are widely used for the treatment of various ailments. *Prosopis cineraria* is a folk remedy for various diseases for major concern. Our results revealed maximum amelioration at 0.04 mg/ml concentration of plant extract under *in vitro* conditions. The study showed that after 2 h of incubation the levels of biochemical and oxidative stress parameters were altered noticeably in samples analyzed after 2 h and 4 h of LPS treatment, in comparison to 0 min, 30 min and 1 h samples. However, the samples retrieved after 8 h and 24 h, the levels of the studied parameters showed marginal difference. The ameliorative effect of bark extract was prominent in the samples retrieved after 2 h incubation. The antioxidant activity of *P. cineraria* is mainly due to the presence of significant amounts of flavonoids, phenolic compounds and thereby, this plant protects from several inflammatory diseases. Flavonoids are known to possess wide array of biological activities, such as anti-oxidative, enzyme inhibiting, apoptosis-inducing, cell-proliferation-inhibiting, and antibacterial [35]. Phytochemical profiling of the crude extracts of the *P. cineraria* unraveled the presence of phyto-constituents-quercetin and apigenin. Quercetin is proven to mediate anti-inflammatory reactions in paw edema induced by carrageenan in rats [36].

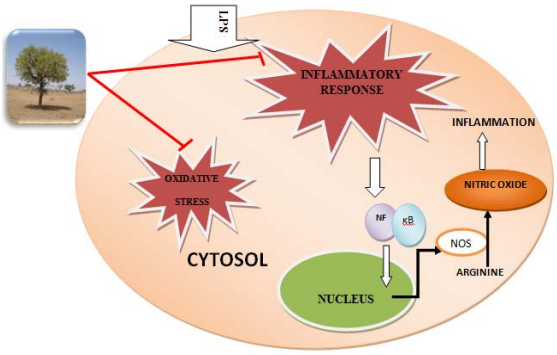

Fig. 1: Diagrammatic Representation of action of *Prosopis cineraria* against LPS-induced inflammation (imaged by Veena Sharma and Preeti Sharma)

It has been postulated that the curative effects of quercetin are due to its antioxidative and free-radical scavenging potential, thereby restoring the oxidative stress mechanisms under the inflammatory status [37]. Moreover, quercetin has been validated to inhibit both proliferation and activation of macrophage under *in vitro* conditions by blocking the activation of lipopolysaccharide-induced nuclear factor B (NF-κB) signalling [38]. Similar to flavonoids, apigenin has been shown to exert anti-inflammatory potential by lowering

oxidative stress though prevention of the expression of several inflammatory factors [39]. It is reported that polyphenols exert anti-inflammatory effects. Since, bark extract of *P. cineraria* is rich in polyphenols such as tannins and flavonoids, the anti-inflammatory effect of plant extract may be rendered due to the presence of polyphenolic compounds [40].

CONCLUSION

In vitro studies are indispensable tool to carry out research for understanding the underlying mechanism with minimal use of animals. The present research work unravelled the alleviating potential of hydro-ethanol extract of *Prosopis cineraria* against LPS-induced toxicity by combating oxidative stress under *in vitro* environment. The alteration in the studied parameters were observed at 0.050 mg/ml LPS concentration; but administration of hydro-ethanol plant extract at concentration 0.04 mg/ml effectively reduced its level when compared to LPS treated samples under *in vitro* conditions. *Prosopis cineraria* is a store house of various phytoconstituents like tannins, steroids, flavone derivatives (namely Prosogerin A, B, C and E), Rutin, Patulitrin, Luteolin, Patuletin, alkaloids etc. which possess anti-oxidative and anti-inflammatory potential and thereby rendering curative role against LPS-induced cell damage. Elaborate *in vivo* studies can be carried in future in order to validate the *in vitro* findings and also, to isolate and characterize such potent active principles present in the stem bark extract of *Prosopis cineraria* which would help in developing herbal formulation for the treatment of inflammation and sepsis.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

Declared none

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