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Original Research
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Isolation, Characterization and Elucidation of Invigorative Potential of Flavonoid From Stem-Bark of *Prosopis cineraria* on LPS-induced Oxidative Stress and Inflammatory Cascade in Swiss Albino Male Mice

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Abstract

The present research aimed to elucidate the structure and characterize the isolated compound from stem-bark of *Prosopis cineraria* and unravel its potential against LPS-induced toxicity in mouse model. The spectral techniques were done for characterization and structure elucidation of the isolated compound (HPLC, NMR, FT-IR, LC-MS. The experimental mice were intoxicated (intra-peritoneal) with LPS (2 mg/kg body weight) and further treated with isolated compound from *Prosopis cineraria* (15 mg/kg body weight). Dexamethasone was used as a standard (10 mg/kg body weight). The oxidative stress parameters (LPO, CAT, SOD, GSH, GST and GPx) and biochemical activities (AST, ALT, ACP and ALP) were studied. The levels of pro-inflammatory cytokines (TNF-α; Prostaglandins E2; IL-6; NF-κBp65; IFN-γ and IL-10) were determined in liver homogenate. Nitric Oxide (NO) produced due to LPS-intoxication was determined by using Griess reagent. The results of the spectral analysis were used to elucidate the structure of the isolated flavonoid. The isolated flavonoid suppressed the over-expression and altered levels of oxidative parameters and cytokines due to LPS intoxication and restored the levels of TNF-α, NF-κB, NO, IL-6, IFN- , Prostaglandin E2 and IL-10. The research investigation unfolded the alleviating potential of the isolated compound against LPS-induced adverse effects by modulating the expression of cytokines and combating oxidative stress.

Keywords: Anti-inflammatory, Cytokines, Flavonoids, Oxidative Stress, *Prosopis cineraria*, ROS

1. Introduction

Despite appreciative research advancements in the field of medical sciences like immunology, oncology, toxicology, pharmacology still India is grappling with various serious diseases like septic shock or endotoxin shock. Sepsis is often referred as a life-threatening multi-organ dysfunction rendered by an unregulated host response to microbial infection[1]. If the unregulated host response persists and remains unrecognized, it can lead to septic shock, multiple organ failure and eventually, death. Any type of infectious pathogen can potentially cause sepsis. Development of antimicrobial resistance is a primary element in determining clinical unresponsiveness to the available medical treatment and rapid evolution to sepsis and septic shock. In present scenario it is intricate to estimate the epidemiological burden of sepsis. LPS, the primary cause of endotoxin shock, is a component of the outer layer present on the surface of most gram-negative bacteria, sometimes lead to high mortality[2].

Medicinal plants are reservoirs of novel medicinal therapeutics due to the presence of various bioactive constituents or phytochemicals[3]. The 'king of the desert', commonly known as Khejri (*Prosopis cineraria*) grows predominantly in dry and arid regions. All the parts of the plant are used for numerous medicinal purposes[4]. The plant has significant antioxidative potential and it is active against lipoxygenase enzyme. Antibacterial potential of the plant extracts was shown against- *Escherichia coli, Salmonella typhi, Pseuodomonas aeroginosa, Bacillus subtilis, Staphylococcus aureus* and presence of various bioactive constituents like alkaloids, glycosides, flavonoids, saponins, tannins, steroids and terpenoids was detected[5].

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The hydro-alcoholic extracts of plant parts like leaf and stem-bark of P. *cineraria* were investigated by trypan blue exclusion method and MTT assay to explore the cytotoxic effects against human cancer cell lines such as HeLa and MCF-7. Hence, it was postulated that the plant extracts of the plant possess potent anticancer activity[6].

In recent years it has been witnessed, that the research for drugs derived from plants has increased tremendously, due to the growing usage of plant-based medicines and their worldwide increasing popularity. The available research data on *Prosopis cineraria* provides sufficient experimental support for the clinical development of *P. cineraria*, a potential herb, as adjuvant therapy. *P. cineraria* successfully fulfil the criteria and can be considered for the development of plant-based medicines due to its potent therapeutic potential, and also its easy availability. The pharmacological activities of *P. cineraria* could be associated, towards a possibility of its incorporation into the present healthcare system. Therefore, the present experimental investigation was conducted to elucidate the structure and characterize the isolated compound from *Prosopis cineraria* and unravel its potential against LPS-induced toxicity in mouse model.

2. Material and Methods

2.1 Chemicals and ELISA Kits

Griess Reagent (Catalogue # G4410) and Lipopolysaccharide (from *Escherichia coli* 0111:B4) were procured from Sigma-Aldrich. The ELISA kits were procured from Invitrogen by Life technologies, Invitrogen Corporation. TNF-α ELISA Kit (Catalog # KMC3011); Prostaglandin E2 ELISA Kit (Catalog # KHL1701); IFN-γ ELISA Kit (Catalog # KMC4021); IL-6 ELISA Kit (Catalog # KMC0061); IL-10 ELISA Kit (Catalog # BMS614INST); NF-κBp65(Total) ELISA Kit (Catalog # KHO0371). 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.

2.2 Experimental Plant

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

2.3 Preparation of Plant Extract

The stem bark was dried in the shade and then, it was powdered by mixer grinder. The prepared stem-bark powder (30 g) was packed in a thimble and subjected to soxhlet extraction with Pet ether and then the obtained marc was extracted in the soxhlet apparatus by 80% ethanol. The prepared extracts were evaporated and dried using rotary evaporator and stored in air tight glass jars at 4°C for further experiments. For experimentation purpose, plant extracts were mixed with their respective solvent in order to attain a concentration of 1 mg/ml.

2.4 Phytochemical Screening and Anti-oxidant Property of the Plant Extracts

The phytochemical screening and quantification determination of flavonoids, saponins, tannins, proanthocynidins, phenols were analyzed. DPPH assay and free radical scavenging assays were performed in order to estimate the anti-oxidative potential of the plant extracts.

In-vitro studies were conducted on the different extracts (Pet ether extract, Ethyl-acetate, Chloroform, Ethanol extract, Hydroethanol extract and aqueous extract) of the plant and amongst all of the extracts, hydroethanol (80%) extract was observed to possess strong anti-oxidative activity.

2.5 Column Chromatographic Analysis

The The column chromatography was carried out using sintered glass column (Extractor length, 600 mm with bore size 30 mm; Borosil). The method used for packing the column was 'wet packing method'. Half of the column petroleum ether (non-polar solvent) was filled in and then silica gel soaked in petroleum ether (before 30 min of packing) was continuously poured in column in order to prepare a uniform bed. Then, adsorbed hydro-ethanol extract was added to the column and placed cotton plug on above the adsorbed extract to avoid any damage of extract layer. Each time 20-25 ml of fractions was collected and the homogeneity of the procured fractions was evaluated on TLC with suitable solvents.

2.6 High Performance Liquid Chromatography (HPLC)

The chemical purity of the final compounds and/or intermediates was characterized by High Performance Liquid Chromatography (HPLC) using a Waters Millenium chromatography system with- 2695 Separation Module (Milford, MA).

2.7 FT-IR (Fourier Transform-Infrared Spectroscopy)

Presence of several functional groups in isolated compound was recorded in the range of 4000-1000 cm^{-1} with the help of FT-IR (Bruker, Germany).

2.8 NMR (Nuclear Magnetic Resonance)

1H NMR spectrum was recorded for the isolated compound (BRUKER Advance III 500MHz, Switzerland). Isolated compound was dissolved in; respective solvent and, observed the peak that arisen by applying the magnetic field.

2.9 LC-MS (Liquid Chromatography-Mass Spectrometry)

Liquid Chromatography coupled with Mass Spectrometry (LC-MS) was used to analyze the mass spectrum of isolated compound by using the instrument-Applied Biosystems; API 3000.

3. Experimental Animals

3.1 Selection of Experimental Animal Models

Biomedical research has relied on the usage of mouse models for evaluating basic mechanisms of pathophysiology, novel therapeutic applications and making decisions regarding new drugs for further clinical research. Studies have shown that the mouse models are capable enough in mimicking human inflammatory disorders. Hence, Swiss Albino Male Mice (*Mus musculus*) of out-bred strain were chosen for the research investigation.

3.2 Maintenance of Experimental Mice

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). The IAEC approval no. of the study was BU/BT/402/14-15. Twenty four Swiss Albino adult male mice (*Mus musculus*) were housed in a duly-ventilated animal house facility with 12 h light-dark cycle. The mice were kept in polypropylene cages (six mice per cage) with ambient temperature (25 ± 30˚C) in an air-conditioned room with humidity in the range of 50 ± 15%. Hygienic conditions were ensured in the animal-house facility. The pelleted diet (purchased from Hindustan Lever Limited, India) constituting [Mn- 55.0, Cu- 10.0, Co- 5.0, Zn- 45.0, and Fe- 75.0- metal content in parts per million (ppm) dry weight] was fed to the mice and drinking water *ad libitum* during the course of research study.

3.3 Liver Samples

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

3.4 Preparation of Liver Homogenate

1.6 gm of liver was homogenised 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.

3.5 Treatment Protocol

In the present experimental study, 24 Swiss albino male mice of 3.5-4 months age were randomly selected (weighing approximately 20–30 g) and divided into four groups having six mice each. Group A was control group with six healthy mice and received normal saline only. Group B was LPS treated group, eighteen mice were treated with LPS (2 mg/kg of b.w., intraperitoneal dose) for fourteen days. After fourteen days, the isolated compound (Group C) and standard dose (Group D) were administered to the mice from 15th day and continued to another 7 days. Hence, the duration of the treatment regime was of twenty one days. The different groups of experimental mice and the treatment regime that was followed are mentioned in the Table 1 given below:

3.6 Study of Oxidative Stress and Biochemical Parameters

Lipid peroxidation, (LPO)[7]; Superoxide dismutase (SOD)[8]; Catalase (CAT)[9]; Alanine Transaminase (ALT) and Aspartate Transaminase (AST)[10]; Reduced Glutathione (GSH)[11]; Glutathione S-transferase (GST)[12]; Glutathione Peroxidase (GPx)[13]; Protein estimation[14]; Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP)[15].

3.7 Estimation of Levels of the Cytokines in Liver Homogenate

The directions and procedures for determining the levels of cytokines (TNF-α, IL-10, IFN-γ, Prostaglandin E2, IL-6 and NF-κBp65) mentioned in the manuals of the ELISA Kits were followed. The calculations were done as mentioned in the manuals of the kits.

3.8 Determination of Nitric Oxide (NO)

The NO levels in the liver tissue samples were determined by using Griess Reagent by following the previously reported protocol[16].

3.9 Statistical Data Interpretation

The experimental results were interpreted as mean ± standard deviation of triplicates. The research 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 results with $p<0.001$ were considered to be highly significant and $p<0.05$ were considered to be satisfactory.

Table 1. Different groups of the experimental mice and the treatment regime that was followed

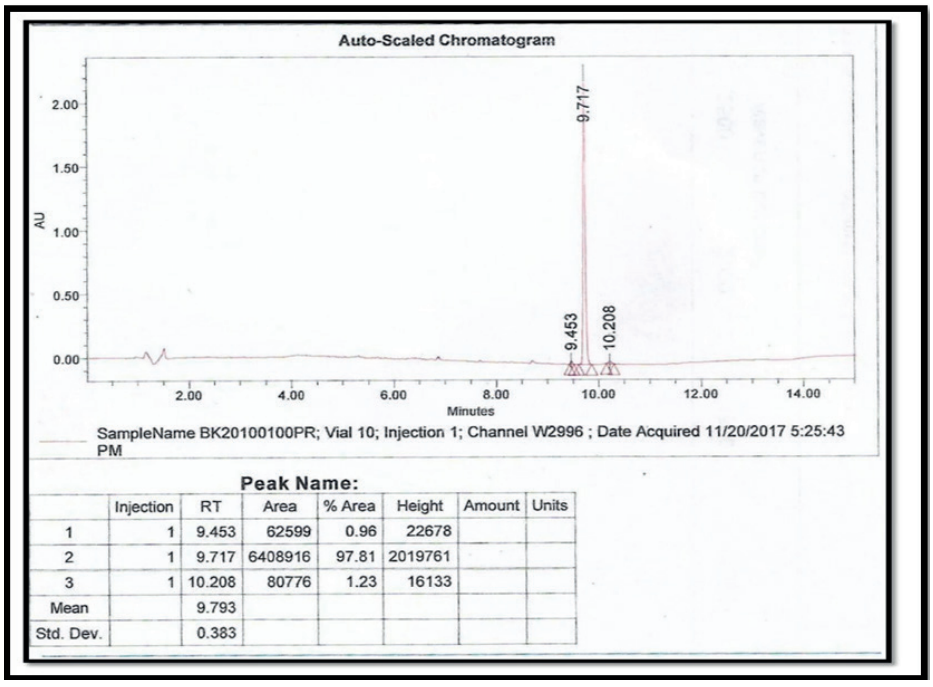
Groups	Treatments	
Group A	Control (n = 6)	Normal Saline
Group B	LPS Treated (n = 6)	2 mg/kg b.w.
Group C	LPS+*Prosopis cineraria* derived Isolated Compound (n = 6)	LPS (2 mg/kg b.w.) + *Prosopis cineraria* derived Isolated Compound (15 mg/kg b.w.)
Group D	LPS+ Standard (Dexamethasone) (n = 6)	LPS (2 mg/kg b.w.)+ Dexamethasone-10 mg/kg b.w.(Standard drug)

4. Results

4.1 Characterization of Compound

The structure of isolated compound was established based on FT-IR, ^{1}H-NMR and mass spectral studies. (Figure 1; Figure 2; Figure 3; Figure 4 and Figure 5).

Loading sample (Isolated compound-5 mg/ml) was prepared in HPLC grade methanol solvent and 10 ul of sample was injected in the column (Zorbax SB; C18- with 150 × 4.6 mm size and 5 µm pore size) and analyzed by UV detector. Wavelength range from 250 to 280 nm was used. Methanol:water (2.5:7.5 v/v) HPLC grade solvent system was used as a mobile phase. Flow rate of sample was maintained at 1.5 ml/minute.HPLC chromatogram of separated compound that showed its Rt 9.7, height 2019761 and relative area 97.81% that is also showed the purity of the compound (Figure 1).



	Injection	RT	Area	% Area	Height	Amount	Units
1	1	9.453	62599	0.96	22678		
2	1	9.717	6408916	97.81	2019761		
3	1	10.208	80776	1.23	16133		
Mean		9.793					
Std. Dev.		0.383					

Figure 1. HPLC chromatogram of the isolated flavonoid.

4.2 FT-IR Spectral Data

The band observed at the region of 3416 cm^{-1} is due to the occurrence of OH stretching frequency of rings A and C. The band present at the region of 2920 cm^{-1} character-

izes the presence of anti-symmetric "C-H stretching" of rings (mainly A, C and D). The aromatic carbonyl group (C = O) stretching attached to ring D; is indicated by the band at the region of 1716 cm^{-1}. The bands at 1437 cm^{-1} and 1504 cm^{-1} indicate "C-C stretching" in nucleus (phenyl) of rings- A, C and D; and -C-O-C- of acyclic 6-membered ring B; respectively. The bands at 1242 cm^{-1} and 1082 cm^{-1} regions indicate "C-H stretching" of E and "C-O-C stretching" of ring C; respectively. The aromatic nature of the rings (A, B) is shown by the presence of bands found at 933 cm^{-1}. The presence of dis-substituted benzene (with C-H bending vibrations) is confirmed by the bands within the region of 900–650 cm^{-1} (Figure 2).

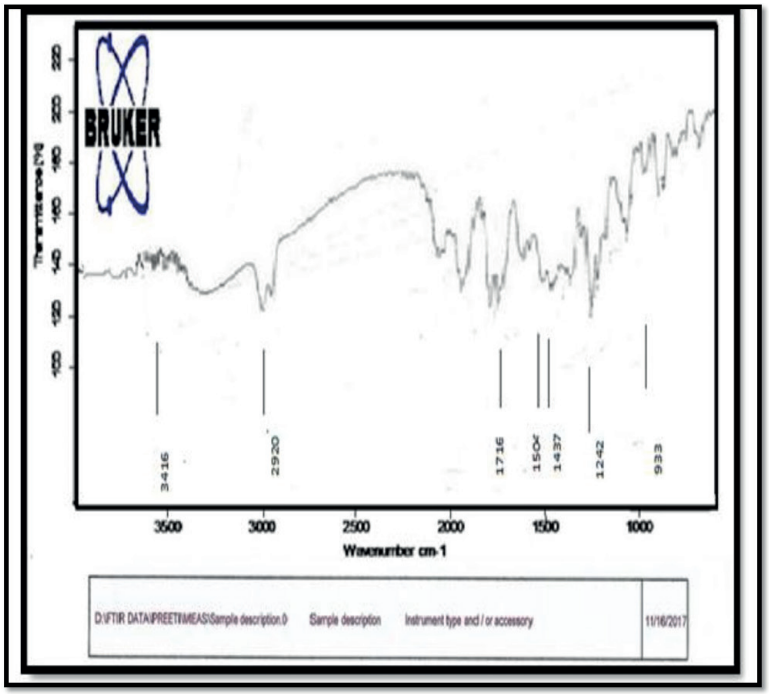


Figure 2. FT-IR spectral data of the isolated flavonoid.

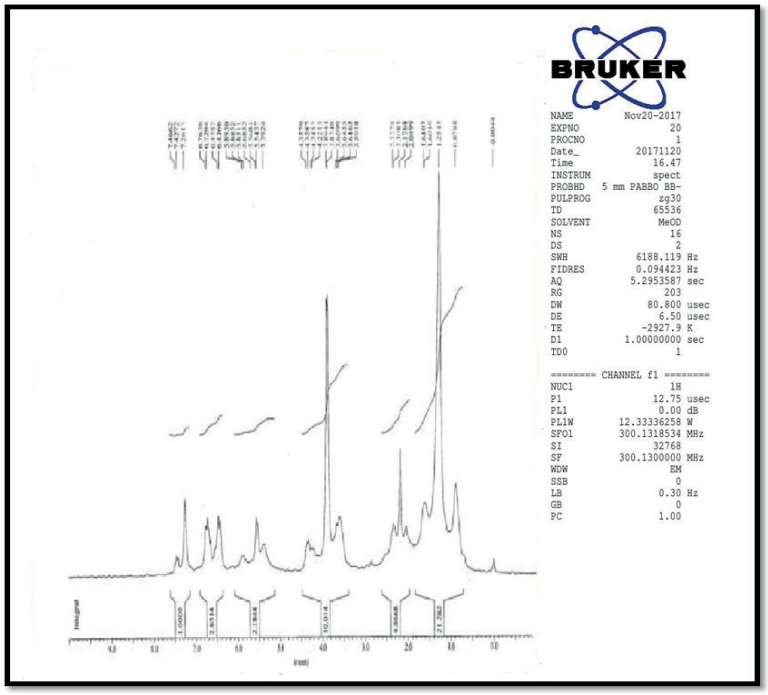


Figure 3. ^{1}H NMR data of the isolated flavonoid.

4.3 ^{1}H NMR Data

^{1}H NMR recorded the multiplet at 7.4 and 7.2 depicts the occurrence of phenolic hydroxyl protons of rings (A and C) and the multiplet at 6.7 and 6.4 shows that the rings (A, C and D) are aromatic. The triplet at 5.39 region is due to the H-1(-O-CH) of ring B. The multiplet at 4.3 regions is because of H-1(-O-CH2) of E. The multiplets at 3.8 and 3.6 regions, signify the occurrence of – CH2-CH-O and H-2 of ring B, respectively. Methoxyl protons of ring D are indicated by the singlet in the region of 2.3. The multiplets at 0.87, 1.25 and 1.6 regions confirm methyl protons, -CH2-R and -CH2 of E (Figure 3).

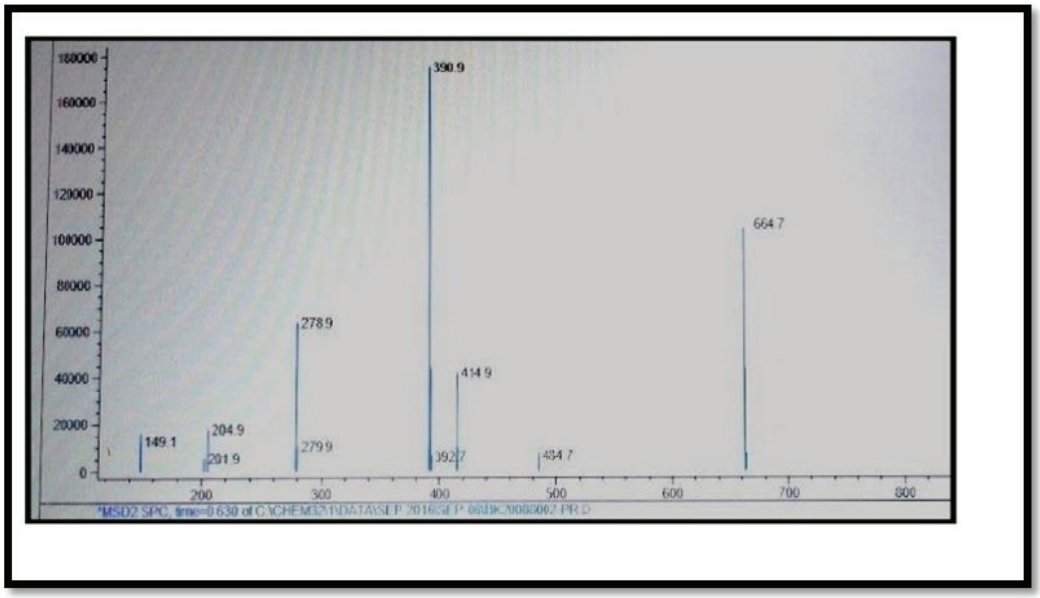


Figure 4. 1LC-MS spectral data of the isolated flavonoid.

4.4 LC-MS (Liquid Chromatography-Mass Spectrum) Spectral Analysis

LC-MS spectrum data of the isolated compound has been observed by Electrospray Ionization method (ESI). In ionization process, sample was dispersed by electrospray into a fine aerosol. The molecular weight was observed by "TOFMS ES- m/z" as 664.7 calculated for $C_{38}H_{48}O_{10}$ and the base peak was observed at 390.9 (Figure 4).

The proposed chemical structure of the isolated compound is given below and the IUPAC name of the isolated compound is "2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one-3-*Undecyloxy-3-galloyl trimethyl ether*" (Figure 5).

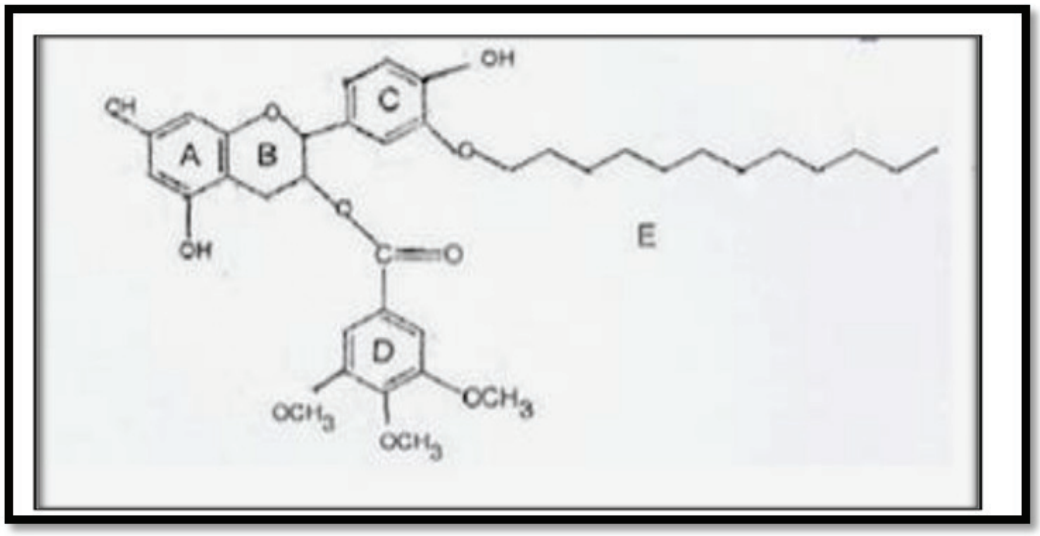


Figure 5. Structure of the isolated flavonoid.

4.5 Oxidative Stress and Biochemical Parameters

All the experimental results of the research investigation of oxidative stress and biochemical parameters are tabulated in Tables 2 and 3. Various oxidative stress markers (i.e. LPO, CAT, SOD, GSH, GST and GPx) and biochemical markers (AST, 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. Administration of LPS into mice led to elevation in the levels of Lipid Peroxidation (LPO) in contrast to control (group A) but phyto-therapeutic treatment of isolated compound reduced its level compared to LPS treated mice (group B). Dexamethasone treated mice also showed reduced LPO levels.

Results also showed decreased SOD, CAT and TPC in group B (LPS treated) in comparison to healthy mice. SOD, CAT and TPC were positively affected by the treatment of isolated compound from *P. cineraria* and Dexamethasone (standard drug). SOD activity was increased in group C and group D in comparison to LPS intoxicated group. The isolated compound has capability to restore CAT activity. LPS intoxicated mice also showed in comparison to control group A, reduced levels of GSH content, GST and GPx activities. The isolated compound administered mice (group C) showed increased GSH content, GST activity and GPx in comparison to LPS treated mice (group B) (Table 2). The mice treated with Dexamethasone (group D) also restored the altered levels of GSH content, GST and GPx.

The experimental results of oxidative stress markers are summarised in the Table 2 given below:

Table 2. Representation of experimental results of oxidative stress parameters

Parameter Analyzed	Control	LPS	LPS+PCIC	LPS+STD
LPO (nmoles of MDA formed/mg tissue)	11.13±0.043	39.05±0.06*	15.07±0.83	12.05±0.078*
SOD (U/min/mg protein)	0.03±0.01	0.012±0.016*	0.019±0.008	0.025±0.0008**
CAT (µmole of H202 consumed/ min/mg protein)	162.04±0.05	124.02±0.72*	146.02±0.95[a]	178.09±0.03[a]
GSH (µmole GSH/g tissue)	3.25±0.02	2.39±0.46*	3.09±0.07	3.76±0.06[b]
GST (nmole CDNB-GSH conjugate formed/ min/mg protein)	512.04±0.35	315.03±0.82**	395.09±0.95[a]	413.07±0.14[a]
GPx (nM NADH oxidised/mg protein	21.06±0.87	11.04±0.38*	19.01±0.04	19.03±0.12[a]
Total protein (mg/g)	13.76±0.43	13.45±0.32	13.95±0.13	13.89±0.11[b]

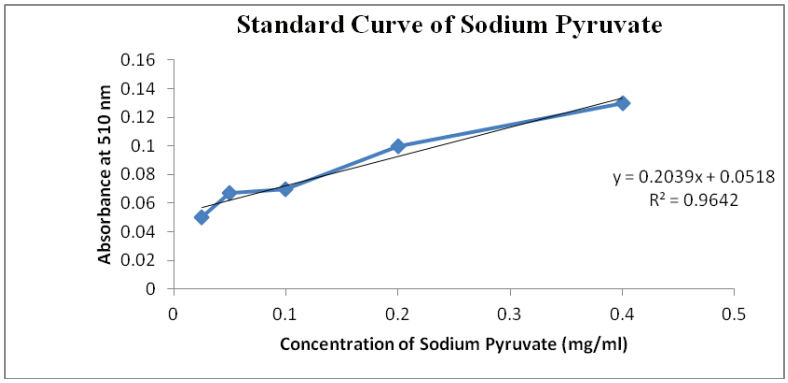
*Values are represented as mean±SD (n = 6). *p<0.05, **p<0.001 v/s control group; [a]p<0.05, [b]p<0.001 v/s LPS treated group. (**LPO**-Lipid peroxidation; **SOD**- Superoxide dismutase; **CAT**- Catalase; **GST**- Glutathione-s- transferase; **GPx**- Glutathione peroxidase; **GSH**- Reduced glutathione; **PCIC** – Prosopis cineraria Isolated compound); **STD**- Standard Drug- Dexamethasone.*

The results highlighted the ameliorative role of isolated compound from *Prosopis cineraria* against LPS induced hepatic toxicity by measuring several "hepato-toxic markers" i.e. AST (Aspartate Amino Transferase), ALT (Alanine Amino Transferase), ACP (Acid Phosphatase) and ALP (Alkaline Phosphatase) (Table 3).

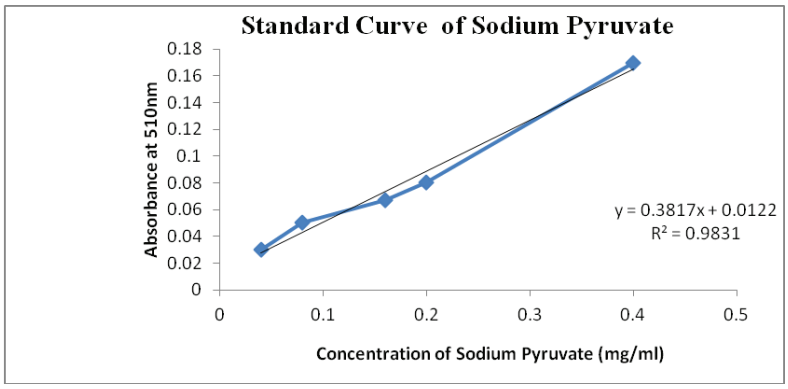
Table 3. Representation of experimental results of biochemical parameters

Parameter Analyzed	Control	LPS	LPS+PCIC	LPS+STD
AST (µmole Pyruvate formed/ min/mg protein)	178.08±0.05	223.03±0.013	197.09±0.15	188.54±0.02[a]
ALT (µmole Pyruvate formed/ min/mg protein)	355.04±0.06	395.03±0.18**	357.51±0.14	347.05±0.12[b]
ACP (µmole pNP released/min/mg protein)	4.49±0.02	3.71±0.014*	4.17±0.09*	4.54±0.09[a]
ALP (µmole pNP released/min/mg protein)	1.84±0.064	2.65±0.023*	2.01±0.08[b]	1.86±0.06[a]

*Values are represented as mean±SD (n = 6).*p<0.05, **p<0.001 v/s control group; ap<0.05, bp<0.001 v/s LPS treated group. (**AST**- Aspartate Transferase; **ALT**- Alanine Transferase; **ACP** –Acid Phosphatase; **ALP**- Alkaline Phosphatase)* **PCIC** *– Prosopis cineraria Isolated Compound);* **STD**- *Standard Drug, Dexamethasone.*



(A)



(B)

Figure 5. Standard calibration curve of sodium pyruvate for AST **(A)** and ALT **(B)**.

AST and ALT activities in liver samples of all treated groups were calculated by using regression equation y = 0.203x+ 0.051; R^2 = 0.964 and y = 0.381x + 0.012; R^2 = 0.983 respectively that were obtained by standard calibration curves of sodium pyruvate (Figure 6). Both AST and ALT activities were increased after LPS intoxication (group B) in comparison to normal mice (group A). The isolated compound (group C) exhibited curative potential to alleviate both AST and ALT activities in liver tissues. Dexamethasone (standard drug) treated mice (group D) showed lower levels of AST and ALT in comparison to group B (Table 3).

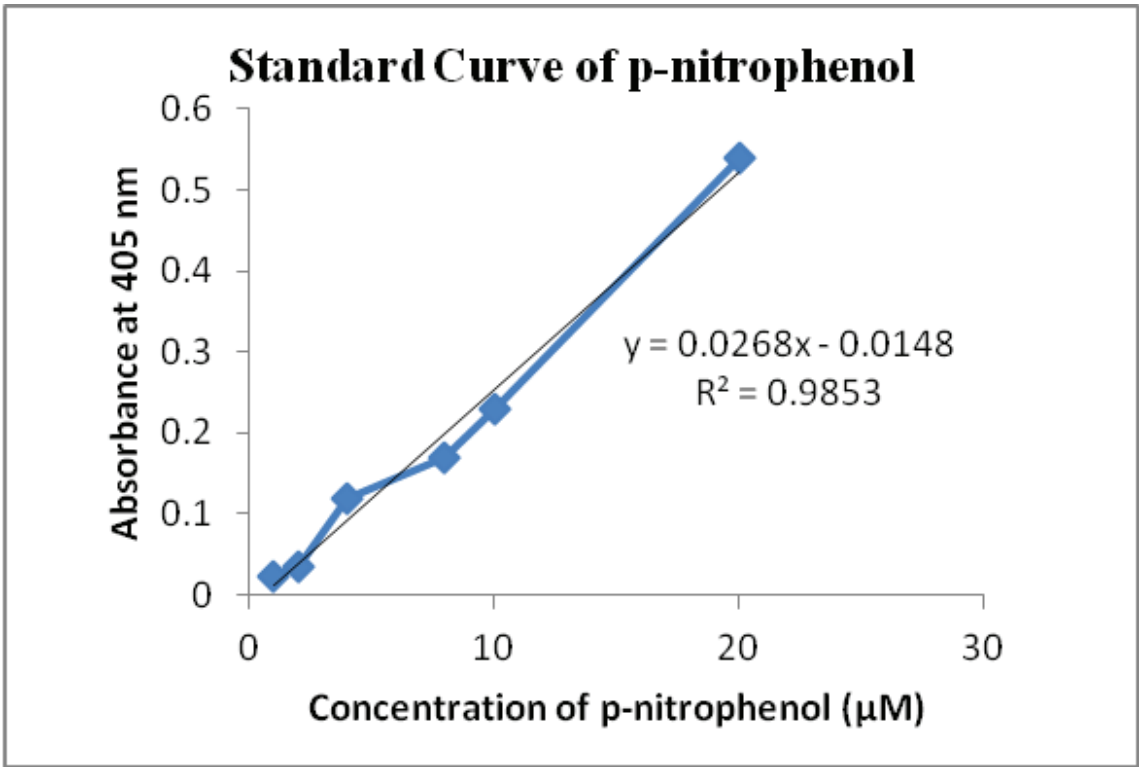
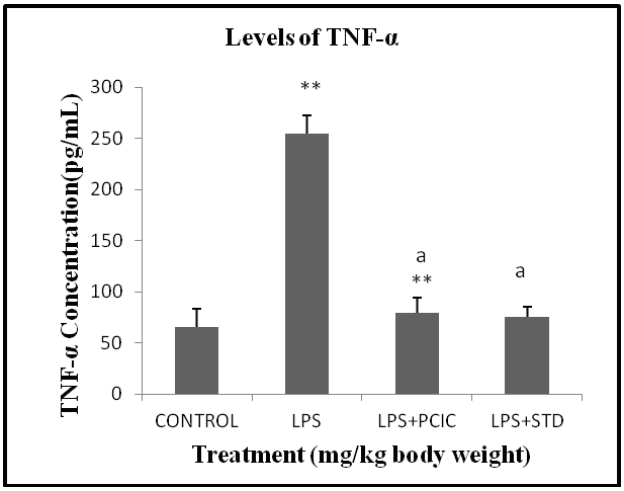


Figure 7. Standard curve of p-nitro phenol.

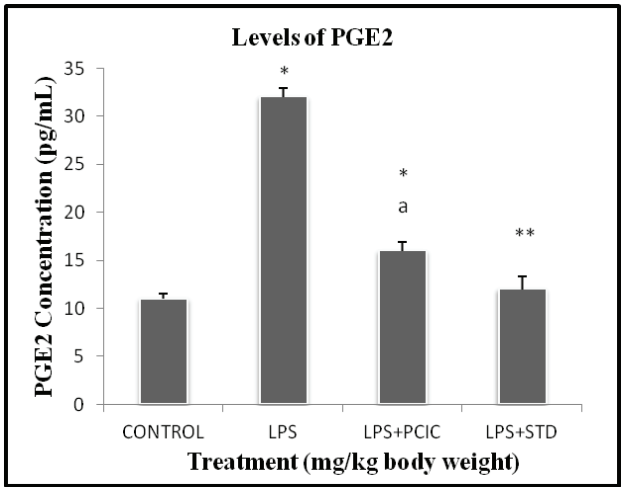
ACP and ALP activities in liver tissue samples of all the treated groups were calculated by using regression equation y = 0.026x + 0.014; R^2 = 0.985 that was obtained by standard calibration curve of p-nitrophenol (pNP) (Figure 7). ACP activity was found to be decreased in hepatic tissues of LPS intoxicated mice in comparison to healthy one (group A). Elevation in hepatic ACP level was observed after administration with the isolated compound (group C) and Dexamethasone (group D).

Results stated that ALP activity was increased in LPS intoxicated mice (group B) when compared to control (group A). The isolated compound showed little potential to normalize ALP level in contrast to Dexamethasone (Table 3).

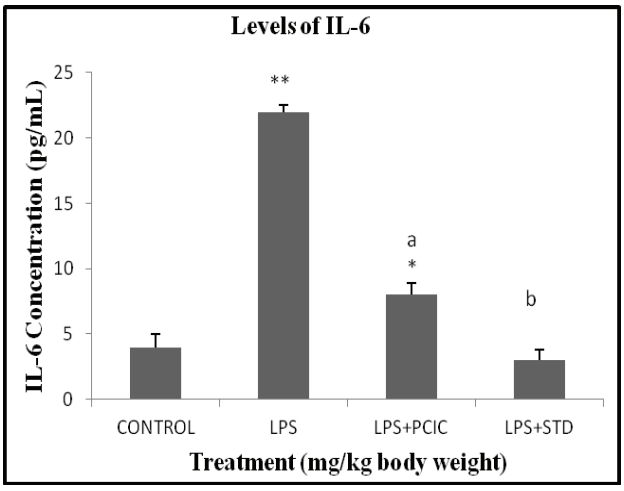
To unravel the anti-inflammatory potential of *P. cineraria*, the activity of key cytokines (namely; TNF-α, IL-6, Prostaglandin E2, IL-10 and IFN-γ) was analyzed. The results showed that LPS caused rise in the TNF-α, Prostaglandin E_2, IL-6 and IFN-γ levels in LPS-intoxicated group in comparison to control group. On the contrary, IL-10 levels were lowered down in LPS-treated mice in contrast to control mice. Quantitative measurements revealed that the isolated compound significantly restored the LPS-induced altered levels of the cytokines. The standard drug (Dexamethasone) also suppressed the elevated levels of TNF-α, Prostaglandin E_2, IL-6 and IFN-γ (Figure 8).



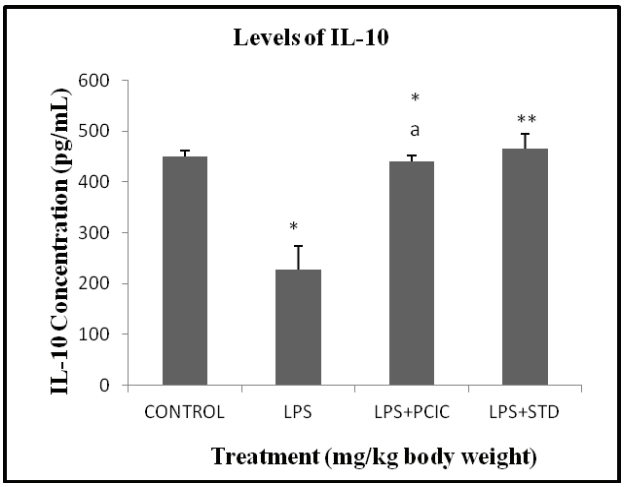
(A)



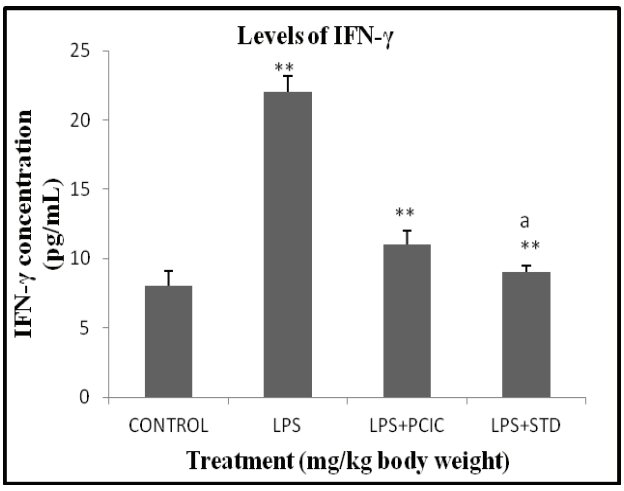
(B)



(C)



(D)



(E)

Figure 8. Graphical representation of levels of Cytokines: **(A)** TNF-α, **(B)** PGE2 **(C)** IL-6, **(D)** IFN-γ and **(E)** IL-10 in Liver tissues of male Swiss albino mice. *PCIC–Prosopis cineraria* Isolated Compound. Values are represented as mean ± SD (n = 6). *p<0.05, **p<0.001 v/s control group (C); ap<0.05, bp<0.001 v/s LPS treated group.

The research study undertaken aimed to detect and quantify the levels of NF-κBp65 protein, independent of its phosphorylation state. NF-κB is reported as a mediator of inflammatory responses, cell division and regulation of apoptosis. Result outcomes illustrated that the levels of NF-κB were elevated in LPS-intoxicated group in contrast to control group (Figure 9). The elevated levels of the protein were lowered down by the action of isolated compound. The standard drug (Dexamethasone) also suppressed the elevated levels of NF-κB.

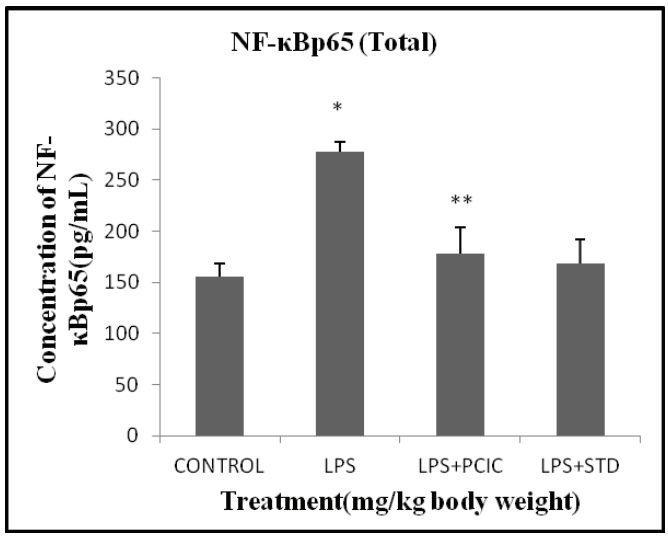


Figure 9. Graphical representation of levels of NF-κBp65 (Total) in liver tissues of male Swiss albino mice. *PCIC– Prosopis cineraria* Isolated Compound. Values are represented as mean ± SD (n=6). *p<0.05, **p<0.001 v/s control group (C);ap<0.05, bp<0.001 v/s LPS treated group.

The levels of Nitric Oxide generated in the liver tissue samples were estimated in the present research investigation. It was observed that the levels of Nitric Oxide were highly enhanced in LPS-intoxicated group in contrast to control group (Figure 10). The altered levels were restored by the administration of isolated compound and Dexamethasone.

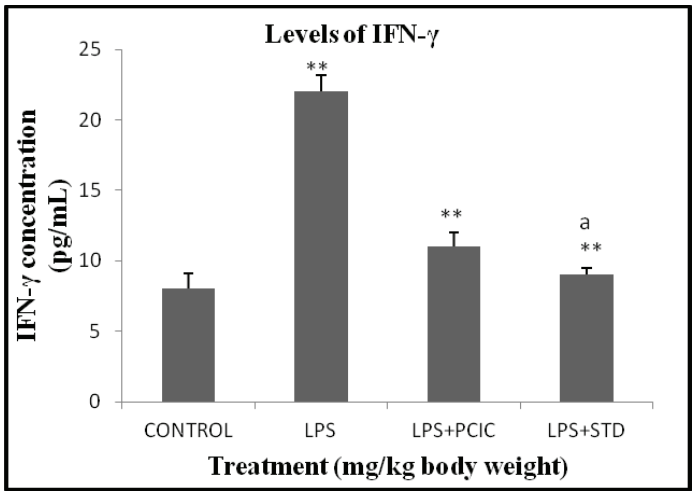


Figure 10. Graphical representation of levels of Nitric Oxide in liver tissues of male Swiss albino mice. *PCIC– Prosopis cineraria Isolated Compound. Values are represented as mean ± SD (n=6). *p<0.05, **p<0.001 v/s control group (C);ap<0.05, bp<0.001 v/s LPS treated group.*

5. Discussion

The focus of the present study was the investigation of the deleterious effects of LPS-induced hepatic toxicity and ameliorative effects of the isolated flavonoid from *Prosopis cineraria* and standard drug, Dexamethasone. The liver is well-known to play a crucial and pivotal role in regulating the entry and metabolism of LPS. The isolated compound from plant is a popular application to reverse and alleviate hepatic injury and oxidative stress generated by LPS and these curative effects may be ascribed to their anti-oxidative and anti-inflammatory potential. LPS intoxication leads to marked increase in lipid peroxidation, which is considered as an important action in triggering the manifestation of various serious disorders. Lipid peroxidation occurs in the presence of some ROS and it is a chain-reaction which is self-propagating, the oxidation of lipids can cause cell damage[17]. LPO subsequently result in the "breakdown of lipids and in the formation of primary oxidation and secondary products" including MDA, which can further react with thiol, amino groups and the deterioration can be extended to even distant sites. SODs carry out the defensive action against "ROS-triggered" injury.[18]. The present research study unravelled the ability of isolated compound in restoring LPO and modulating oxidative stress. Since the isolated compound is a herbal flavonoid, it possess ability to directly bind RONS and scavenging them or by acting as antioxidants thereby, inhibiting the lipid peroxidation cascade as noticed in this study. The experimental findings showed that there is a decrease in SOD levels in the LPS-treated group. SOD can inhibit neutrophil-mediated inflammation and may restore the ROS-dependent tissue injury.[19] Extracellular Superoxide Dismutase, Mn-SOD and Cu, Zn-SOD have been considered as "potent inhibitors" of inflammatory cascade by researchers[20–22].

In the present experimental study, catalase activity against LPS-induced toxicity was analyzed. Altered catalase activity renders disrupted removal of ROS.[23]. Overexpression of catalase enzyme has been demonstrated to alleviate ROS-induced cell damage[24] while lower levels of catalase promote autophagic cell death.[25]. 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. Glutathione Peroxidase (GPx) 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[26]. Previously published report suggests that the LPS intoxication leads to severe oxidative stress and significant depletion in the levels of GSH[27]. Since, ROS-mediated oxidative imbalance have been clearly studied that could be responsible for hepatic toxicity induced by LPS, hence, ALT activity was much higher than the AST activity in hepato-toxic conditions as observed after LPS intoxication in mice. ALP (Alkaline Phosphatase) and ACP (Acid Phosphatase) 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)[28]. The alteration in ALP activity is linked with the damage in membrane permeability that leads to cell damage.

Studies revealed that increase in "oxidative stress" is linked with activation of NF-κB. Exposure to hydrogen peroxide leads to the nuclear translocation of NF-κB and generation of various pro-inflammatory cytokines which can be curbed by the activity of anti-oxidants[29]. NF-κB signals are a central regulator of inflammatory cytokines, which are key factors in inflammation responses[30]. *Prosopis cineraria* showed considerable anti-inflammatory effect against LPS induced inflammation in mice. Results unravelled that the levels of NF-κB were significantly higher in LPS-treated mice and there was an associated increment in the levels of inflammatory mediators such as IL-6, IFN-γ and TNF-α. The levels of NF-κB were reduced in the isolated compound treated group and standard drug treated group. The levels of other cytokines such as IL-6, IFN-γ and TNF-α were also lowered down. These observations are in the agreement with the previously published reports[31].

Up-regulated generation of cytokines in LPS-induced mice is a known mechanism and study has shown that the increased levels of pro-inflammatory factors in the liver were correlated to liver injury. The present research work proposes that the mechanism for the witnessed decline in the levels of TNF-α, IL-6 may be ascribed to the involvement of the ability of "anti-oxidative nature of the phyto-actives present in the isolated compound" in the process of inhibition of the "Kupffer cells activation" by LPS. This eventually leads to inhibiting the mechanism of TNF-α synthesis and subsequently IL-6 synthesis is also hindered. As a matter of fact, NF-kB, transcription factor, involved in the regulation of several other genes which play categorically vital role in inflammatory responses in the host and stimulation of numerous cellular signalling pathways, which target and trigger the mass generation of inflammatory cytokines[32]. LPS intoxication in the host leads to marked increase in the liberation of Nitric Oxide (NO) and Superoxide anion (O_2•-) free radicals during activation of Kupffer

cells[33]. These findings suggest the possible protective role of the isolated flavonoid. So, this study revealed that isolated flavonoid from the hydroethanol extract of the stem bark of P. cineraria may provide host defence by exerting anti-inflammatory and wound healing response. This assumption is significantly correlated with previous studies on various extracts of *P. cineraria*, including aqueous extract[34-35]. Anti-inflammatory activity has been exhibited by the water-soluble extract obtained from the residue of methanolic extract of *P. cineraria* [36-37]. The antioxidant activity of *P. cineraria* is mainly due to the "possession of significant amounts of flavonoids, phenolic compounds" and thereby, this plant protects from several inflammatory diseases. It is reported that polyphenols exert anti-inflammatory effects. Since, the isolated compound from *P. cineraria* is a flavonoid, the anti-inflammatory effect of the plant may be rendered due to the presence of polyphenolic compounds[36]. Flavonoids are known to possess wide array of biological activities, such as anti-oxidative, enzyme inhibiting, apoptosis-inducing, cell-proliferation-inhibiting and antibacterial[38]. Phytochemical profiling of the crude extracts of the *P. cineraria* unravelled the presence of phyto-constituents-quercetin and apigenin. Quercetin is proven to mediate anti-inflammatory reactions in rats[39]. It has been postulated that the curative effects of quercetin are due to its antioxidative and free-radical scavenging potential[39]. Moreover, quercetin has been validated to block the activation of LPS-induced nuclear factor- κB (NF-κB) signalling[40]. Similar to flavonoids, apigenin has been shown to exert anti-inflammatory potential by lowering the expression of several pro-inflammatory factors[41].

6. Conclusion

The in-vivo pharmacological studies involve induction and treatment of liver toxicity with isolated flavonoid from *P. cineraria*. The administration of isolated flavonoid showed restoration capability when compared to standard drug Dexamethasone. In the experimental studies, these findings suggested that the oral administration of the isolated flavonoid possess anti-inflammatory activity against LPS-induced hepatic toxicity as validated by the significant and dose dependent restoration of biochemical and oxidative stress parameters. Decline in the levels of Lipid Peroxidation (LPO) and SOD (Superoxide Dismutase), while increase in the levels of antioxidant enzymes (CAT, GST, GSH, GPx) via scavenging of free radicals, or by enhancing the activity of antioxidant, which than detoxifies the free radicals. These factors protect cells from ROS-trigerred damage in LPS-induced hepatic toxicity. The research findings demonstrated that the isolated flavonoid modulated the levels of cytokines. The stem-bark extract of *P. cineraria* possess abundant quantity of phytochemicals and antioxidants. These antioxidants play a pivotal role in inhibiting the transfer of "NF-κB" to nucleus, thereby curbing the enormous generation of inflammatory mediators. These observations and description of mechanism of the isolated flavonoid would possibly lead to rapid development in sepsis treatment.

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8. Conflict of Interest

There is no conflict of interest.

9. References

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