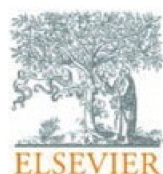

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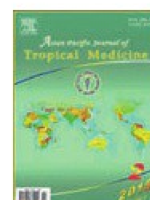

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Cytoprotective and anti-inflammatory effects of kernel extract from *Adenanthera pavonina* on lipopolysaccharide-stimulated rat peritoneal macrophages

Arunagirinathan Koodalingam[1*], Ramar Manikandan[2], Munisamy Indhumathi[1], Ethala Subramani Kaviya[1]

[1]*Department of Biotechnology, Sri Sankara Arts and Science College, Enathur, Kanchipuram-631 561, Tamilnadu, India*

[2]*Department of Animal Health and Management, Alagappa University, Karaikudi-630 003, Tamilnadu, India*

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ABSTRACT

Objective: To investigate mechanism of anti-inflammatory activity of *Adenanthera pavonina* (*A. pavonina*) extracts. **Methods:** Rat peritoneal macrophages were treated with different concentrations of lipopolysaccharide and H_2O_2 in the presence and absence of kernel extract from *A. pavonina*. Nitric oxide, superoxide anion generation, cell viability and nuclear fragmentation were investigated. **Results:** The pre-treatment of kernel extract from *A. pavonina* suppressed nitric oxide, superoxide anion, cell death, nuclear fragmentation in lipopolysaccharide and H_2O_2 stimulated or induced macrophages, respectively. **Conclusions:** These results suggest that *A. pavonina* extract suppresses the intra cellular peroxide production.

1. Introduction

Inflammation is an adaptive response that is triggered by toxic stimuli and conditions such as infection and tissue injury[1]. The initial recognition of infection is mediated by tissue resident macrophages. Macrophages are considered to play essential roles in inflammation and if activated by endotoxin, it produce inflammatory mediators including vasoactive amines, lipid mediators, pro-inflammatory cytokines, chemokines, proteolytic enzymes and reactive oxygen/nitrogen species, which have been implicated in the pathogenesis of tissue injury[2-4]. Lipopolysaccharide (LPS), a component of the Gram-negative bacteria cell wall, is well-known as an effective stimulus in activation of macrophages to secrete pro-inflammatory cytokines and secondary mediators such as nitric oxide (NO) and superoxide anion[5].

NO production is mediated by the inducible NO synthase (iNOS), which is a member of the NO synthase (NOS) family. NO is a short-living free radical that is produced from L-arginine by catalytic reaction of NOS within mammalian immune, cardiovascular and neural system, where it functions as a signaling or cytotoxic molecule[6]. The iNOS is the key enzyme involved in NO production by macrophages stimulated by bacterial endotoxin of LPS and proinflammatory cytokines such as interferon-gamma and tumor necrosis factor-alpha[6,7]. Activity of the iNOS is regulated at different levels from transcriptional and post translational steps[8-10]. Therefore, blocking of macrophage functions inclusive of inflammatory mediators may have a therapeutic potential in the treatment of inflammatory diseases.

Recently, the rising interest in various natural compounds from oriental medicines which have potentials for treatment of inflammatory diseases has led to increased attention to

*Corresponding author: A. Koodalingam, PhD., Assistant Professor, Department of Biotechnology, Sri Sankara Arts and Science College, Enathur, Kanchipuram-631 561, Tamilnadu, India.
Tel: 044-27264066 (Extn: 19)
E-mail: akoodalingam@gmail.com

their potential safety and efficacy. *Adenanthera pavonina* (*A. pavonina*) is a common tree that belongs to the family Fabaceae, and it is found worldwide and chiefly known for its bright red color seeds. Various parts of this plant have been used in Indian traditional medicine. The plant *A. pavonina* has been reported to possess antibacterial, antioxidant, anthelmintic, anti–hyperlipidemic, blood pressure lowering and anti–inflammatory effects[11–16]. The presence of anti–inflammatory property is particularly useful in the prevention of arthritis and various neurodegenerative diseases like Alzheimer's disease, where inflammation mediated excess production of oxidative free radical plays a key role in development of such diseases. Only two preliminary studies have been performed in carrageenan induced rat paw edema model and reported the presence of anti–inflammatory property in bark and seed extracts of *A. pavonina*. Previous *in vivo* anti–inflammatory studies provide an important clue to select this plant for the further study to get its effect on macrophages. However, no report was available to access its *in vitro* anti–inflammatory effect on any of the immune cells, especially macrophages. Therefore, the present investigation was undertaken to evaluate the anti–inflammatory and cytoprotective response in kernel extract of *A. pavonina* on LPS stimulated macrophages.

2. Materials and methods

2.1. Fine chemicals

LPS (*Escherichia coli*), 1–napthyl ethylenediamine dihydrochloride, sulfonilamide and nitroblue tetrazolium chloride were from Sigma Chemical Company (St. Louis, MO, USA). Propidium iodide (PI), RPMI–1640 medium, fetal bovine serum, 2.5 g/L trypsin–EDTA, and antibiotics were purchased from Himedia Laboratories (Mumbai, India). All other chemicals and reagents used in this study were of analytical grade and commercially available.

2.2. Animal maintenance

Male albino rats (100–150 g) were procured from the National Institute of Nutrition (Hyderabad, India). All experiments were approved by the Institutional Animal Ethical Committee guidelines (IAEC 360/01/a/CPCSEA). Animals were housed in an air–conditioned room at (22±10) ℃ with a lighting schedule of 12 h light and 12 h dark. Rats were fed on a balanced commercial rat diet (Hindustan UniLever, Mumbai, India) and water was given *ad libitum*.

2.3. Extract preparation

The seeds of *A. pavonina* were collected from Guindy Campus, University of Madras, Chennai. The seed coat of the dried seeds was manually removed and the resulting kernel was fine powdered using a mixer grinder. The pulverized fine powder was used for the preparation of aqueous and methanol extracts. For aqueous extract preparation, 10 g of fine powder was suspended in 100 mL of distilled water and stirred well with a magnetic stirrer at 15 ℃ overnight. The mixture was centrifuged at 10 000 r/min at 4 ℃ for 20 min. The clear supernatant was filtered using Whatman filter paper, lyophilized and stored at –20 ℃ for further use. The stock solution was prepared by dissolving the powder in RPMI medium.

For methanol extract preparation, 30 g of fine kernel powder was extracted with 250 mL of methanol using Soxhlet apparatus. The extract was evaporated to dryness using a vacuum evaporator to yield a 3.69 g residue. In order to prepare stock solution, 250 mg of residue recovered from the methanol extract was dissolved in 2 mL of DMSO. From this stock solution, various concentrations of the extract were prepared by suitably diluting the stock with RPMI medium.

2.4. Harvesting culture of peritoneal macrophages

Peritoneal macrophages were harvested from a healthy rat using the method described by Davies and Gordon[17]. The harvested macrophages were cultured in RPMI–1640 medium, washed once with fresh medium and transferred to 75 cm^2 tissue culture flask containing RPMI–1640 medium + 10% (v/v) fetal bovine serum along with antibiotics and kept for 2 h in an incubator at 5% (v/v) CO_2 atmosphere. After 2 h, the culture flask was observed under inverted phase contrast microscope (Optika XDS, Italy) to visualize the adhered macrophages. The non–adhered cells, mainly erythrocytes and a small number of lymphocytes, were removed by washing the flask 3 times with Hank's balanced salt solution. After ensuring complete removal of the non–adhered cells, fresh RPMI–1640 medium supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin and 50 μg/mL streptomycin was added and maintained in a humidified incubator with 5% CO_2 (v/v) at 37 ℃.

2.5. MTT assay for determination of cell viability

The cell viability of macrophages were determined with 3–4,5–dimethylthiazol 2–4,2,5–diphenyltetrazolium bromide (MTT) reduction assay by the method described by Wang *et al*[18]. Briefly, the macrophages were pre–incubated overnight in 96–well plates at a density of 2×10^5 cells per well and then washed with PBS (pH 7.5) to remove fetal bovine serum. Cells with various concentrations of aqueous (1.250, 2.500, 5.000 and 10.000 mg/mL) and methanol extracts (0.125, 0.250, 0.500 and 1.000 mg/mL) were treated with H_2O_2 as well as LPS for 24 h, and then they were cultured in MTT (0.5 mg/mL) at 37 ℃ for 4 h. After the culture supernatants were removed, the resulting dark blue crystals were dissolved with dimethyl sulfoxide (DMSO). Absorbance

values were read at 540 nm on an ELISA plate reader (Bio–Rad, USA). All determinations were performed in duplicates and confirmed at least three independent experiments.

2.6. Estimation of NO generation in rat macrophages

The nitrite concentration in the culture medium supernatant was quantified by colorimetric assay as an indicator of NO production based on the Griess reaction by Jung *et al*[19]. Briefly, 2×10^5 cells were plated and incubated with the aqueous or methanol extract of *A. pavonina* at various concentrations for 2 h. After LPS (1.0 μg/mL) stimulation for 24 h, a sample of peritoneal macrophage cell culture medium was saved for the measurement of nitrite. Then, 500 μL of culture supernatant from the untreated or treated medium was mixed with an equal volume of Griess reagent consisting of 250 μL of 1 g/L 1–napthyl ethylenediamine dihydrochloride and 250 μL of 10 g/L sulfanilamide, followed by incubation at room temperature for 20 min. The absorbance of the mixture was measured at 570 nm in an ELISA plate reader (Bio–Rad, USA) against a suitable reagent blank. The level of NO produced was calculated using the standard graph of sodium nitrite and expressed as μm nitrite released.

2.7. Estimation of superoxide anion generation in rat macrophages

The generation of superoxide anion by macrophages was quantified spectrophotometrically with nitroblue tetrazolium (NBT) method described by Vidya *et al*[20] with suitable modifications. Briefly, macrophages were cultured on sterile cover glass (2×10^5) which was placed inside the wells of 24 well tissue culture plates and incubated in a CO_2 incubator overnight. After that, the cells were washed with PBS followed by addition of aqueous or methanol extracts, left for 2 h and washed once again with PBS. In order to induce inflammation, LPS (1 μg/mL) in serum free medium was added and incubated for another 24 h. Then, 0.5 mL of 1 g/L NBT was added and incubated for 45 min. The enzyme reaction was stopped by addition of 0.5 mL of 100% (v/v) methanol. The samples were recovered and centrifuged at 10 000 r/min at 4 ℃ for 10 min, and the supernatants were decanted. The resulting pellet was mixed with 2 mL of extraction fluid to dissolve the NBT reduction product, blue colored formazan. Then the samples were centrifuged at 4 000 r/min at 4 ℃ for 10 min, and optical density (OD) of the clear supernatants was measured in a spectrophotometer (Cecil CE 7200) against suitable reagent blank at 625 nm. The amount of generated superoxide anion was expressed as OD at 625 nm.

2.8. Fluorescence microscopic evaluation of cell death

To further examine cytoprotective effects of aqueous and methanol extracts of *A. pavonina* on macrophages, H_2O_2 induced cell death was evaluated under Labomed FLR fluorescence microscope using DNA staining PI. Cell death was assessed by the uptake of the fluorescent exclusion dye PI. This fluorescent dye is impermeable to cells with intact plasma membrane because of efflux mechanisms, whereas it easily enters and stains the nucleus of dead cells. PI has its maximum excitation at about 530 nm and emission at 615 nm. Briefly, macrophages were cultured on sterile cover glass (1×10^4 cells in each cover glass) which was placed inside the tissue culture plate and incubated overnight in CO_2 incubator. After that, the cells were washed with PBS followed by addition of aqueous or methanol extracts and left for 2 h. Control slides received PBS or DMSO. The monolayer was washed with PBS to remove the extracts (to prevent direct interaction between compound and H_2O_2) prior to the addition of H_2O_2. The cell death was induced by the addition of 50 μmol/L H_2O_2 in RPMI medium and incubated for 1 h in a moist chamber. After 1 h, the monolayer was once again washed with PBS to remove H_2O_2. Subsequently, 200 μL of 1 g/L PI in PBS was added and incubated for 10 min to allow the dead cells to take the dye. The monolayer was gently washed with PBS to remove the excess dye and place cover glass. The slides were observed under the fluorescence microscope (Labomed FLR, USA).

2.9. Statistical analysis

The mean differences between control and experimental values were calculated using unpaired student's *t*–test. Each experiment was performed 4–6 times using samples from different preparations. The data are represented as mean±standard deviation. $P>0.05$ was considered nonsignificant in all instances.

3. Results

3.1. Effect of A. pavonina on macrophage cell viability

Using MTT assay, we first investigated the effect of aqueous and methanol extracts of *A. pavonina* on cell viability of the isolated rat peritoneal macrophages. As shown in Figure 1, exposures to the aqueous extract at four different concentrations for 24 h caused little effect on the macrophages. This observation clearly revealed that the aqueous extract at the highest test concentration (10.000 mg/mL) produced 10% reduction of cell viability, whereas the extract at the lower concentrations did not affect the viability and the extract was safe for the macrophages. The viability of macrophages treated with the methanol extract at concentrations of 0.125 and 0.250 mg/mL for a 24 h period was reduced by 3% and 4% compared with that of control. When treated with the extract at the concentration of 0.500 mg/mL, 6% reduction in the cell viability was observed. However, exposure to methanol extract (1.000 mg/mL) caused a statistically insignificant reduction (10%) in macrophage

viability (Figure 1).

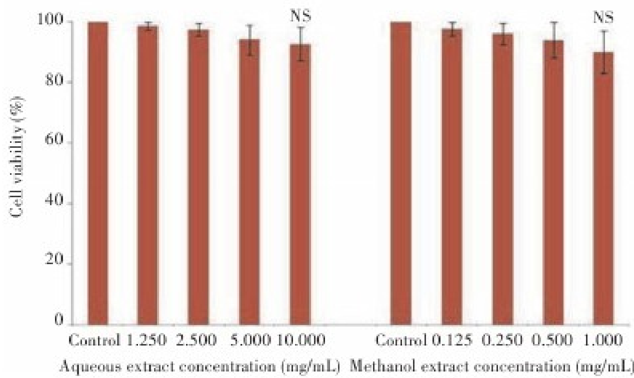

Figure 1. Effect of *A. pavonina* extracts on viability of peritoneal macrophages.
NS indicates no statistically significantly difference between control and extract exposed cells.

3.2. Effect of LPS on cell viability of macrophages

The present study was performed to ascertain the effect of LPS on macrophage cell viability. As presented in Figure 2, LPS at all the four tested concentrations (0.5–10.0 μg/mL) failed to show any impact on macrophage cell viability. These results clearly proved that the tested concentrations of LPS only induced the inflammation in macrophages, as evidenced by increased production of NO without effect on cell viability. It has been reported that short term exposure (24 h) of macrophages to LPS results in no loss of cell viability.

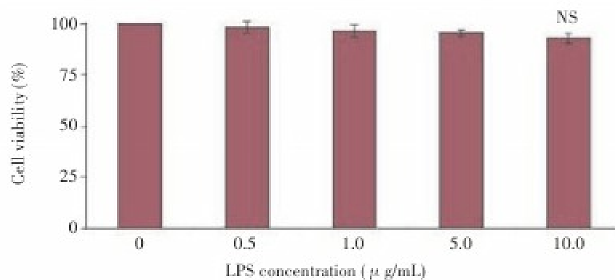

Figure 2. Effect of LPS on viability of peritoneal macrophages.
NS indicates no statistically significantly difference between control and LPS exposed cells.

3.3. Effect of LPS on NO production in macrophages

To evaluate the inflammatory conditions of macrophages upon stimulation with bacterial endotoxin LPS, NO production was measured with Griess reagent. As shown in Figure 3, LPS at three different concentrations significantly induced the production of high amount of NO in macrophages, indicating that the resting macrophages were activated by LPS and it thereby secreted a number of inflammatory mediators including NO. The lowest test concentration (0.5 μg/mL) had the ability to induce 8 fold over control. A dose dependent increase of NO production was observed in macrophages upon stimulation with LPS. There was no significant difference between the higher concentrations of LPS (1.0–5.0 μg/mL) as they stimulate a fairly stable amount of NO. Therefore, 1.0 μg/mL LPS was subsequently selected to induce inflammation in macrophages.

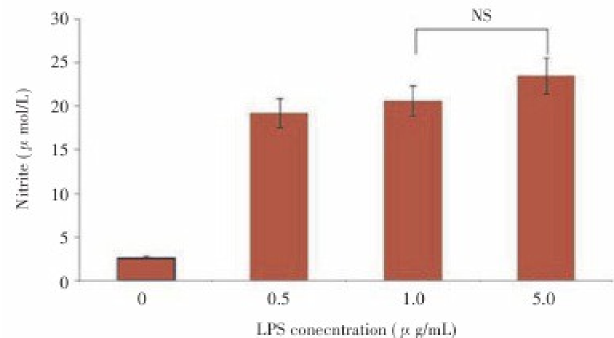

Figure 3. Effect of LPS on NO generation in peritoneal macrophages.
NS indicates no statistically significantly difference between 1.0 and 5.0 μg/mL LPS concentrations.

3.4. Effect of A. pavonina extracts on NO production in LPS-stimulated macrophages

To determine whether *A. pavonina* regulates the production of NO on LPS (1.0 μg/mL) induced inflammatory conditions, the macrophages were pre-treated with the aqueous extract for 2 h. As shown in Figure 4, the pre-treatment of the aqueous extract of *A. pavonina* significantly reduced the amount of NO as measured by the stable end product nitrite. A dose dependent decrease in the amount of nitrite production was also observed. Moreover, the pre-treatment of macrophages with the methanol extract of *A. pavonina* also reduced the LPS mediated generation of NO in rat macrophages. The tested concentrations of 0.5 and 1.0 mg/mL significantly inhibited the NO production to 11.6 and 6.8 μmol/L nitrite ($P<0.001$). However, exposure of macrophages to methanol extract dose-dependently decreased the NO production in rat macrophages (Figure 5).

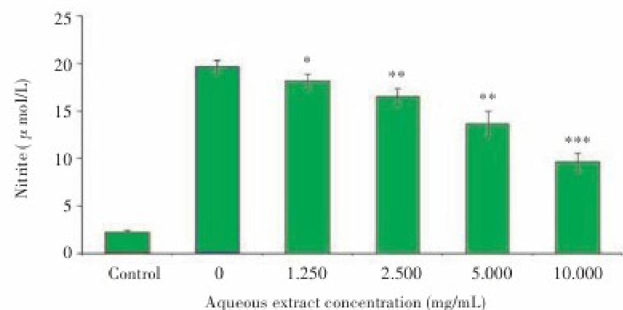

Figure 4. Effect of aqueous extract of *A. pavonina* on LPS induced NO generation in peritoneal macrophages.
Asterisk indicates statistically significant difference between untreated control (LPS alone) and extract + LPS treated cells (*$P<0.05$; **$P<0.002$; ***$P<0.001$).

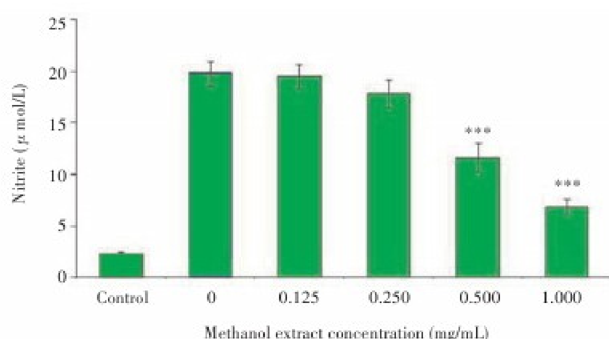

Figure 5. Effect of methanol extract of *A. pavonina* on LPS induced NO generation in peritoneal macrophages.
Asterisk indicates statistically significant difference between untreated control (LPS alone) and extract + LPS treated cells (***P<0.001).

3.5. Effect of *A. pavonina* extracts on superoxide anion generation in LPS–stimulated macrophages

Many independent investigations support the view that oxidative stress is the main responsible factor for many diseases. The production of reactive oxygen species like superoxide anion is one of the key factors in inflammation mediated cell damage. In order to investigate whether extracts of *A. pavonina* has the inhibitory potency against LPS stimulated superoxide anion generation in macrophages, NBT assay was performed. Macrophages were pre–treated with the aqueous or methanol extract of *A. pavonina* followed by exposure to LPS (1.0 μg/mL). High level of superoxide anion generation was observed in the untreated control, indicating that LPS had stimulated reactive oxygen species in rat peritoneal macrophages. The exposure to both the extracts significantly inhibited the superoxide anion generation in a dose dependent manner (Figures 6 & 7). Among the two extracts tested, the methanol extract exhibited potent inhibitory activity against LPS stimulated superoxide generation in rat macrophages.

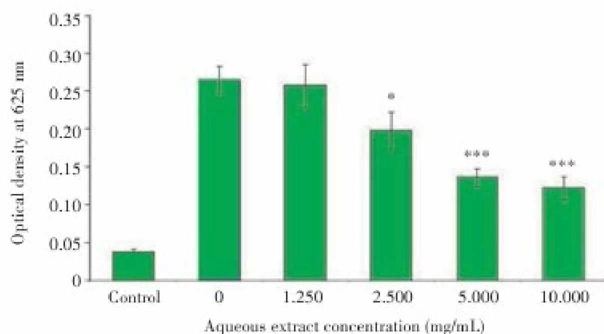

Figure 6. Effect of aqueous extract of *A. pavonina* on LPS induced superoxide anion generation in peritoneal macrophages.
Asterisk indicates statistically significant difference between untreated control (LPS alone) and extract + LPS treated cells (*P<0.05; ***P<0.001).

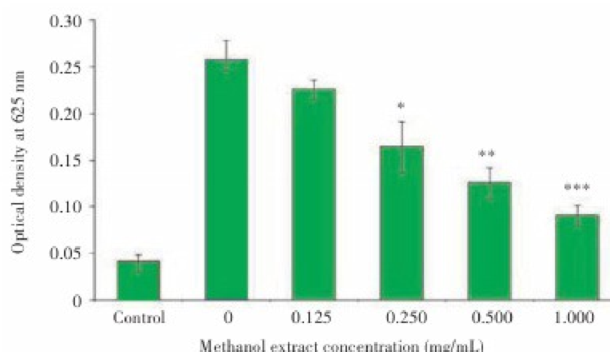

Figure 7. Effect of methanol extract of *A. pavonina* on LPS induced superoxide anion generation in peritoneal macrophages.
Asterisk indicates statistically significant difference between untreated control (LPS alone) and extract + LPS treated cells (*P<0.05; **P<0.002; ***P<0.001).

3.6. Effect of H_2O_2 on macrophage cell viability

In order to evaluate the possible cytoprotective properties of the *A. pavonina* extracts on macrophages, we first studied the effect of H_2O_2 on the cell viability in cultured peritoneal macrophages. As shown in Figure 8, the exposure to H_2O_2 significantly reduced the cell viability as shown by MTT assay. A dose dependent reduction of cell viability was observed upon exposure to H_2O_2. One hour exposure to 50 μmol/L H_2O_2 exhibited about 50% cell death in macrophages (P<0.001). However, the low concentration of H_2O_2 (1 μmol/L) produced about 11% cell mortality. Moreover, the higher concentration of H_2O_2 (100 μmol/L) was able to knock down 90% of the macrophages within 1 h of incubation (P<0.001). To assess the cytoprotective role of both extracts of *A. pavonina*, cells w–ere pre–treated for 2 h. After that, cell death was initiated by the addition of 50 μmol/L H_2O_2. This test concentration was selected for further cytoprotective experiments, because it was able to produce 50% cell death in macrophages at this level.

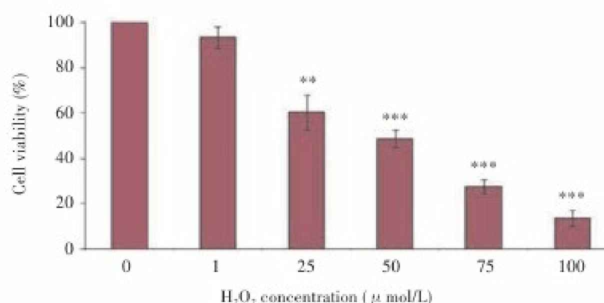

Figure 8. Effect of hydrogen peroxide on viability of peritoneal macrophages.
Asterisk indicates statistically significant difference between untreated control and H_2O_2 treated cells (**P<0.002; ***P<0.001).

3.7. Fluorescence microscopic evaluation of cytotoxicity

The cytoprotective effect of *A. pavonina* extracts on H_2O_2 induced cell toxicity in macrophages was evaluated using fluorescent microscope. PI is a fluorescent stain that selectively stains the DNA molecule of dead cells. The number of stained cells in a single microscopic field is proportional to the severity of cytotoxic nature of the molecule. As shown in Figure 9A, no cell death was observed in the control. However, large numbers of stained cells were observed in the macrophages exposed to 50 μ mol/L H_2O_2, clearly indicating that the exposure of H_2O_2 brought out the production of cell death on macrophages (Figure 9B). It is interesting to note that a less number of stained cells were observed in the *A. pavonina* extracts pre–treated cells (Figure 9D, 9E and 9F). The results of the present study revealed that the exposure to *A. pavonina* extracts attenuated the H_2O_2–mediated cytotoxicity in macrophages.

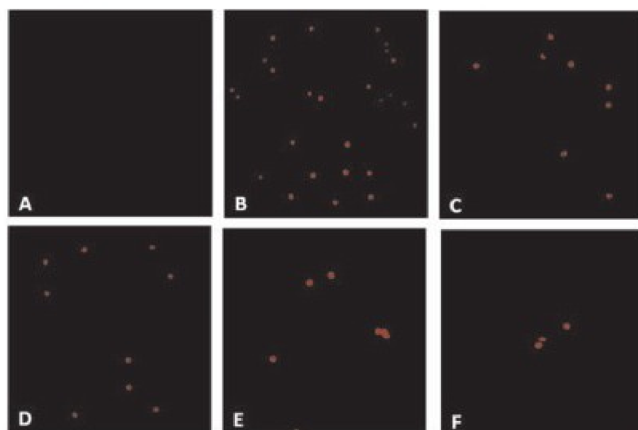

Figure 9. Cytoprotective effect of *A. pavonina* extracts on macrophages (40× objective lens).
A: Control; B: Pretreatment of cells with 50 μ mol/L H_2O_2; C: Pretreatment of cells with aqueous extract (5.0 mg/mL); D: Pretreatment of cells with methanol extract (0.5 mg/mL); E: Pretreatment of cells with aqueous extract (10.0 mg/mL); F: Pretreatment of cells with methanol extract (1.0 mg/mL).

4. Discussion

There is much importance in exploring the potential pharmacological effects of plant compounds against many inflammatory mediated human diseases[21]. The present study was undertaken to examine the protective effects of extracts from *A. pavonina* on the induction of NO production and superoxide anion generation in LPS stimulated rat macrophages. To further understand the cytoprotective effect of *A. pavonina* on macrophages, cell viability was also observed. The results of the present investigation revealed an anti–inflammatory effect by inhibiting LPS stimulated production of NO and superoxide anion generation. Besides, it also exhibited cytoprotective activity by attenuating H_2O_2–mediated cell death in peritoneal macrophages of rats. Few investigators have reported the toxicity of plant compounds on mammalian cells. Recently, curcumin at the concentration of 20–25 μ mol/L was found to significantly affect the viability of cultured MES 23.5 cells, whereas that at low doses (up to 15 μ mol/L) is safe for the cells[18]. In the present study, both the extracts caused little effect on the viability of rat macrophages. Among the two extracts tested, the aqueous extract exhibited less toxicity at low concentrations than the methanol extract of *A. pavonina*.

Macrophages play a vital role in inflammatory response by releasing a number of inflammatory mediators. The signaling events that occur during inflammatory processes have been fully established and they help to recruit more immune cells to sites of infection or tissue–injury[22,23]. Bacterial LPS has been shown to activate the inflammatory signaling events by binding to macrophage cell surface receptor that result in induction through iNOS via activation of NF–κB[2,24]. We have reported that short term LPS exposure (24 h) did not affect cell viability of rat macrophages, but maybe chronic exposure of LPS would have exhibited cytotoxicity as reported[25]. LPS–stimulated macrophages rapidly produce potent mediators such as reactive oxygen species and nitrogen intermediates[24,26]. The overproduction of these reactive molecules is harmful to the nearby cells and results in development of many inflammatory and autoimmune diseases[27–29]. Therefore, NO inhibition through pharmacological interference has the potential therapeutic option to control many inflammatory mediated human disorders. A number of studies have demonstrated that plant compounds exhibit anti–inflammatory property by inhibiting NO production[30,31]. Many investigators reported that the pre–treatment of macrophages with botanicals inhibit NO production. Glycyrol isolated from plant *Glycyrrhiza uralensis* inhibited NO production in LPS stimulated RAW264.7 macrophages, curcumin and its hydrogenated metabolites down regulate the production of NO in macrophages, ethanolic extract of *Pimpinella anisoides* and its constituents reduces oxidative damage by inhibiting NO in LPS stimulated RAW264.7 macrophages[32–34]. In the present study, a dose dependent decrease in the NO level was observed in macrophages treated with the *A. pavonina* extracts. The results revealed that the methanol extract of *A. pavonina* exhibited high inhibitory activity against NO production than the aqueous extract.

Reactive oxygen metabolites, especially superoxide anion, produced by inflammatory cells are toxic to the nearby cells and in combination with NO produce more toxic peroxynitrite[35]. The polymorphonuclear leucocytes have the ability to generate NO and superoxide anion upon stimulation with carrageenan[36]. Therefore, reducing the generation of superoxide anion in macrophages would help better to combat the inflammation. As recently reported, pre–treatment with *Acanthopanax senticosus* significantly inhibit the LPS induced intracellular peroxides, thereby reducing the oxidative stress of RAW264.7 macrophage cell line[26]. We have reported that the pre–treatment with

aqueous and methanol extracts of *A. pavonina* attenuates the generation of superoxide anion in a dose dependent manner, thereby reducing oxidative stress in LPS stimulated rat peritoneal macrophages.

The excess production of H_2O_2 upon inflammatory stimulus leads to death of brain cells and attributes to involvement of oxidative stress mediated cell death in development of ischemia and neurodegenerative diseases[37–39]. Brain cells, especially astrocytes, produce high amount of reactive oxygen species in the form of H_2O_2 that has been implicated to the destruction of cholinergic nerve cells as observed in the pathogenesis in Alzheimer's disease[40,41]. H_2O_2 may initiate cell death by combining with intracellular metal ions such as iron or copper, resulting in production of highly toxic hydroxyl radicals[42–44]. As a first step, we assessed the cytotoxicity of H_2O_2 on rat peritoneal macrophages by observing the cell vialbility, and our results revealed that higher concentration was toxic to the cells. Therefore, 50 μmol/L concentration of H_2O_2 which exhibited about 50% cell mortality was selected as an optimal dose for the assessment of the cytoprotective nature of *A. pavonina* extracts on macrophages. When the untreated cells were exposed to exogenous H_2O_2, the number of cells that took up the fluorescent dye increased. Hence, the H_2O_2 enters into the nucleus, damages the chromatin and leads to cell death. However, the pre–treatment of macrophages with the aqueous and methanol extracts of *A. pavonina* protected the cells from H_2O_2 mediated generation of oxidative damage, thereby reducing the number of stained cells. This reduction observed upon treatment may be due to the radical scavenging property of the components present in the extracts of *A. pavonina*. Li *et al.* noted that the pre–treatment with polysaccharide from *Cordyceps sinensis* protect the hydrogen peroxide mediated cell injury in PC12 cells[45]. Subsequently, Chen *et al.* has also reported that the exposure to quercetin (a flavanoid) attenuates the H_2O_2 induced apoptotic cell death in rat glioma C6 cells[46]. Similarly, Matsushima *et al.* have evaluated *in vitro* pharmacological actions of three major phenolic antidiarrheic ingredients, namely, 2M4MP, 2M4EP and 2MP, on H_2O_2 induced oxidative stress, cellular viability in cultured astrocytes and neurons of the rat brain[47]. Our results here are consistent with previous observations and indicate that the excess production of free radicals by the action of H_2O_2 is reduced by free radical scavenging activity of the aqueous and methanol extracts of *A. pavonina*.

The present observation implies that the extracts of *A. pavonina* possess beneficial anti–inflammatory effects by reducing the production of NO and superoxide anion generation in macrophages. Moreover, the extract has cytoprotective property by inhibiting the H_2O_2 mediated generation of oxidative damage in rat peritoneal macrophages. The simultaneous reduction of both reactive oxygen and nitrogen intermediates may be useful to combat against many inflammatory mediated diseases. Besides, its low cytotoxic nature even at higher concentrations and their cytoprotective property give a thrust to detect the active ingredients present in the crude extract which exhibits multifunctional property, and this deserves detailed investigation.

Conflict of interest statement

We declare that we have no conflict of interests.

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