

# Anti-inflammatory and Anti-hyperuricemic Effect of *Ficus benghalensis* Bark Extract in Raw 246.7 Cell Line

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## ABSTRACT

**Background:** Hyperuricemia and inflammation are associated with the etiology and pathogenesis of several metabolic diseases. Controlling the elevated levels of uric acid and inflammation can be a potential therapeutic option for several metabolic diseases.

**Aim:** The current study examined the anti-inflammatory and anti-hyperuricemic properties of *Ficus benghalensis* bark extract *in vitro* using murine macrophage RAW 246.7 cells.

**Materials and Methods:** Hydroalcoholic extracts of *Ficus benghalensis* bark were obtained. Cytotoxicity and cell viability test of the extracts was evaluated by MTT and Trypan blue exclusion assay. Cells were microscopically observed for the presence of dead cells. To explore the anti-inflammatory and anti-hyperuricemic activity, RAW 246.7 cells were induced with lipopolysaccharide and treated at the concentrations of 20, 40, and 60  $\mu\text{g/ml}$  of *Ficus benghalensis* bark extract and assessed for xanthine oxidase activity, uric acid levels, reactive oxygen species, lipid peroxidation assay, nitric oxide levels and gene expression studies of a pro-inflammatory and anti-inflammatory cytokine such as TNF- $\alpha$  and IL- 10. **Results:** A dose-dependent reduction in the xanthine oxidase activity when treated with the bark extract with higher inhibition at 60  $\mu\text{g/ml}$ . Reduction in the amount of uric acid, ROS, nitric oxide, and lipid peroxidation are similar to xanthine oxidase activity with a significant decrease in all treated groups and higher reduction at 60  $\mu\text{g/ml}$  treatment. Gene expression study showed downregulation of TNF- $\alpha$  and upregulation of IL- 10 in treated groups. **Conclusion:** Our study demonstrates that *Ficus benghalensis* bark extract exhibits anti-hyperuricemic and anti-inflammatory effects possibly through xanthine oxidase inhibition. The results provide evidence that *Ficus benghalensis* bark extract may have the potential to treat hyperuricemia and complications related to hyperuricemia.

**Keywords:** Anti-hyperuricemic, RAW 246.7 cells, *Ficus benghalensis* bark extract, Anti-inflammatory, TNF- $\alpha$ , IL- 10, Lipopolysaccharide.

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## INTRODUCTION

Degradation of uric acid yields uric acid. Hyperuricemia is an elevated uric acid level in the blood that may occur either because of overproduction or because of insufficient clearance of uric acid.<sup>1</sup> The normal upper limit of uric acid is 6.8 mg/dL and values over 7 mg/dL is considered to be hyperuricemic.<sup>2</sup> Hyperuricemia has been related to various diseases like chronic renal failure, gout, cardiovascular disease and

metabolic syndrome.<sup>3</sup> Accumulation of uric acid leads to inflammation and oxidative stress in various tissues such as joints and kidney.<sup>4,5</sup> Thus, controlling hyperuricemia and inflammation together serves as the major therapeutic target to relieve from the ill effects of hyperuricemia.

*Ficus benghalensis* is popularly known as the banyan or banyan Figure *Ficus benghalensis* is a fast growing and a large tree that has



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been widely introduced across tropical regions and subtropical regions worldwide. Traditionally various parts of *Ficus benghalensis* has been used to treat various ailments due a variety of secondary metabolites present. Most of these metabolites are polyphenols, flavonoids, steroids, volatile oils and triterpenes.<sup>6</sup>

Few studies explored the anti-oxidant,<sup>7</sup> anti-diabetic,<sup>8-10</sup> antidepressant,<sup>11</sup> wound healing<sup>12,13</sup> and immunomodulatory<sup>14-16</sup> effects of various extracts of *Ficus benghalensis*. However, the anti-inflammatory and anti-hyperuricemic effects of *Ficus benghalensis* bark extract hasn't been previous investigated. Hence, the current investigation aimed at elucidating the potential of hydroalcoholic bark extract in inhibiting xanthine oxidase activity and anti-inflammatory potential.

## MATERIALS AND METHODS

### Preparation of *Ficus benghalensis* bark extract

20 g of dried *Ficus benghalensis* bark powder was soaked in 70% methanol for 48 hr at room temperature with periodic stirring and shaking. After 48 hr, the stirred solvent was filtered using a 125 mm diameter Whatman filter paper. The filtrate was evaporated at 40°C and the dried powder was used for further assays.

### MTT Assay

Effects of *Ficus benghalensis* bark extract (FBBA) was evaluated using a modified MTT assay,<sup>17</sup> MTT is enzymatically reduced to a purple colored compound, formazan. About  $5 \times 10^4$  RAW 264.7 cells were seeded in 96-well plates, induced using LPS (500 ng/mL) and treated with *Ficus benghalensis* bark extract at 10 different concentrations (10 to 100 µg/ml). After the treatment for 24 hr, 15 µl of MTT at 0.5 mg/mL concentration was added to the cells and incubated at 37°C for 4 hr. The supernatant was discarded and to dissolve the formazan, 0.1 ml of DMSO was used. These plates were kept on a shaker for 10 min at room temperature and then the absorbance was determined at 490 nm and 630 nm. The cells in the control group was considered as 100% viable cells. The inhibition rate was determined using the formula:

$$\% \text{ Inhibition Rate} = \left\{ 1 - \frac{A_{490} - A_{630} (\text{Treated})}{A_{490} - A_{630} (\text{control})} \right\} \times 100\}$$

### Trypan Blue Exclusion Assay

Trypan blue exclusion assay was performed to estimate the lethality of *Ficus benghalensis* bark extract on RAW 264.7 cells.<sup>18</sup> RAW 264.7 cells were seeded in 6-well

plates, induced with LPS for a day in an incubator (5% CO<sub>2</sub>, 37°C) and treated with 10-100 µg/ml of *Ficus benghalensis* bark extract. Later, cells were trypsinized and suspended in 10% DMEM (equal volume) and trypan blue. The viable (unstained) and dead cells (dark blue) cells were enumerated using a hemocytometer.

### Phase Contrast Inverted Microscopy

Morphological changes of apoptotic cells observation was carried out as per the protocol in<sup>19</sup> with some modifications.  $5 \times 10^5$  cells were incubated with extract 0, 20, 40, and 100 µg/ml of *Ficus benghalensis* bark extract induced with LPS treated cells for 24 hr in culture dishes (60 mm). The cells were given a PBS wash. The morphology of the cells for apoptotic changes were visualized in a phase contrast inverted microscope at 200x magnifications.

### ROS estimation

The NBT reduction assay was performed for ROS estimation with slight modification from.<sup>20</sup>  $1 \times 10^5$  cells/ml seeded and incubated for 24 hr. Test samples were then added into appropriate wells and incubated for 24 hr. Washed with PBS twice and 100 µl of 0.1 % NBT were added to each well of the 24 hr incubated cells and approximately after an hour the medium was discarded and washed with 70 % methanol thrice. To the dried well 120 µl of potassium hydroxide (KOH) 2 M and 120 µl of DMSO was added to the wells and then measured 630 nm using a plate reader with DMSO or KOH as blank.

### Estimation of Nitrite Concentration

Nitrite concentrations was used to determine the nitric oxide released from the RAW 264.7 using the protocol reported by.<sup>21</sup> After complete confluency, the cells were treated with trypsin and subjected to centrifugation for 10 min at 1000 rpm.  $50 \mu\text{l}$  of  $4 \times 10^5$  cells/well were seeded in a 96 well plate. To enhance the attachment of cells, the plates were incubated at 37°C. Different concentration of the *Ficus benghalensis* bark extract (20, 40, and 60 µg/ml) were supplemented and incubated for 24 hr. Griess assay was used for nitrite determination. Sulfanilamide and NED solution were kept at 25 – 28°C for about 20 min to allow the solutions to equilibrate.  $50 \mu\text{l}$  of the culture supernatant was added to 96 well plate. 1% sulfanilamide (50µl) in 5% H<sub>3</sub>PO<sub>4</sub> was added to all the wells and was incubated 10 min in dark at room temperature. Then, 50µl of 0.1% NED was added to the well and incubated for 10 min in dark place. After incubation, a dark blue color was formed

and the absorbance was taken using a plate reader between 520nm and 550nm.

### Lipid peroxidation assay (LPO)

Thiobarbituric acid-reactive substance (TBARS) assay was performed by the method of<sup>22</sup> in order to determine lipid peroxidation. In this assay, the complex that was formed by the reaction of malonaldehyde with thiobarbituric acid was quantified spectrophotometrically. The treated cells with various concentration of the test sample were taken for the assay. The cells were homogenized with ice cold KCL Buffer then the homogenized tissue sample was collected in Eppendorf Centrifuged for 5 min at 5000g. The pellet was re-dispersed in 200 µl of 8.1 % SDS and then 1.5 ml of 20% AA was further added to this suspension and incubated for 10 min, then 1.5 ml of 0.8 % TBARS buffer and 700 µl of sterile water was added. This mixture was incubated at 95°C for 1 hr and cooled to 25°C. To the reaction mixture, 5 ml of Pyridine and Butanol mixture in the ratio 1:15 was added and subjected to centrifugation at 5000g for 15 min to remove debris. Absorbance was taken at 532 nm with untreated cells were taken as a control. The standard was performed with MDA. The sample values compared with standard and the concentration expressed as µM.

### Xanthine oxidase Activity

Effect of different concentration of *Ficus benghalensis* bark extract (control, LPS induced, 20, 40, and 60 µg/ml) on XO activity. XO catalyze the conversion of xanthine to uric acid with production of superoxide radical (O<sub>2</sub><sup>-</sup>). 2.7 mL of buffer was added into a mixture with 100 µl of xanthine solution and the contents were mixed well, then 100 µl of enzyme solution (sample) was added. The mixture (A1) was spectrophotometrically read at 293 nm and incubated for 30 min. After 30 min, 100 µl TCA were added and centrifuged for 10 min at 5000-x g. The second absorbance value were read at 293 nm (A2) as reported by<sup>23</sup>

Absorbance change (ΔA) was calculated using:

$$A = [(A2) \text{ assay} - (A2) \text{ blank}] - [(A1) \text{ assay} - (A1) \text{ blank}]$$

$$A/\text{min} = A/30$$

### Uric acid estimation

Effect of different concentration of *Ficus benghalensis* bark extract (control, LPS induced, 20, 40, and 60 µg/ml) on Uric acid estimation. Uric acid estimation was done by using *Tulip Diagnostics* Uric Acid Kit (Uricase

- PAP method) in accordance with the manufacturer's instruction.

### DNA isolation

Culture medium was removed from the cells, trypsinized and collected by centrifugation for 5min at 1200 rpm. Supernatant were discarded and the cells were resuspend with 1 to 10 ml ice-cold PBS and centrifuged 5 min at 1200 rpm. The supernatant was removed.  $<3 \times 10^7$  cells was suspended again in 0.3 ml of digestion buffer and incubated at 50°C for 12 to 18 followed by addition of phenol-chloroform-isoamyl alcohol gently mixed and centrifuged at 5000 rpm for 5 min. Without disturbing the middle or bottom layer, the aqueous phase was carefully transferred. Compared to the aqueous layer, double the volume of ethanol and 1/2 the volume of ammonium acetate with 7.5 M was added, and centrifuged for 5min at 5000 rpm. 70% ethanol was gently overlaid on the pellet to rinse it and the ethanol was discarded and air dried. The resulting pellet was dissolved in 30µl of nuclease free water for further processes.

### Gene expression studies

#### Primers

For the current study β-Actin, TNF-alpha and IL-10 primers were used. All the primers was designed by obtaining the nucleotide sequence form GenBank.

#### PCR

PCR was carried out using Thermo cycler gradient PCR. 1 µl of primers, 4.5 µl template DNA, 2.5 µl of PCR master mix and distilled water was added and made up to 25 µl. Gene amplification was performed using 1 cycle of initial denaturation for 2 min at 95°C then 34 denaturation cycles for 1 min at 95°C, 30 sec for annealing at 54°C for β-Actin, 54.6°C for IL-10 and 55.4°C for TNF-α, extension at 72°C for and final extension for 5 min as 72°C. 6 µl of amplified PCR products was mixed 4 µl of gel loading dye (6X) and run on a agarose gel electrophoresis (1% agarose) containing EtBr (1 mg/ml), using 1 X TAE buffer at 50 V for 2 hr. Separated amplicon products were visualized under UV and photographed.

### Statistical analysis

The results obtained in the experiments are expressed in the mean ± standard deviation.  $P < 0.0001$  was generally considered to be significant. The statistical analysis was performed using GraphPad Prism 5

software, Test type: one-way ANOVA (Turkey multiple comparison tests).

## RESULTS

### MTT assay

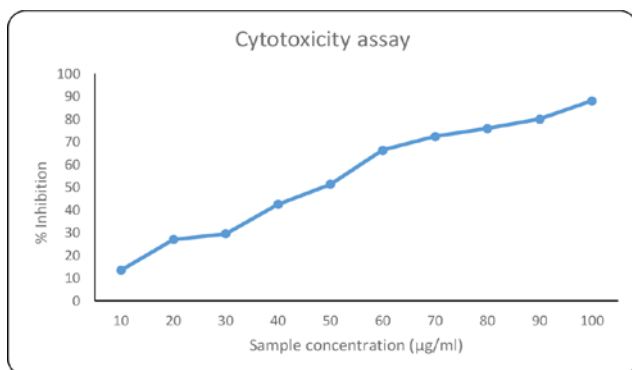
Toxic effect of *Ficus benghalensis* bark extract in Macrophage cells (RAW cells) was studied by MTT assay. The varied concentrations from 10 - 100  $\mu\text{g/ml}$  were showed cytotoxicity effect of *Ficus benghalensis* bark extract in Macrophage cells (RAW 246.7) as shown in the Figure 1. The  $\text{LC}_{50}$  range of the *Ficus benghalensis* bark extract was found at 50  $\mu\text{g/ml}$  concentration with 51.26% inhibition, whereas inhibition increased in dose dependent manner as illustrated in Figure 1.

### Trypan Blue exclusion Assay

The cell viability of Macrophage cell line (RAW 246.7) treated with *Ficus benghalensis* bark extract was studied by trypan blue assay. The percentage viability of *Ficus benghalensis* bark extract at 10 $\mu\text{g/ml}$  was 86.56% and 48.73% at 50 $\mu\text{g/ml}$  compared to untreated cells. The Figure 2 shows percentage of cell viability of RAW 264.7 significantly decreased in dose dependent manner as observed in Figure 2.

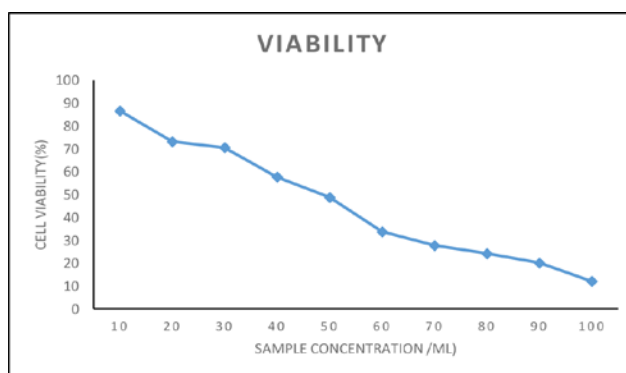
### Morphometric Analysis

Photomicrograph (20 x) represents morphological changes with detachment, shrinkage, membrane blebbing and disoriented cell shape induced by *Ficus benghalensis* bark extract treatment (0, 20, 40 and 100  $\mu\text{g/ml}$  for 24 hr) when compared with control as shown in Figure 3. The cells in control group showed normal cell morphology and was intact. The images were captured by phase contrast microscope (Figure 3).



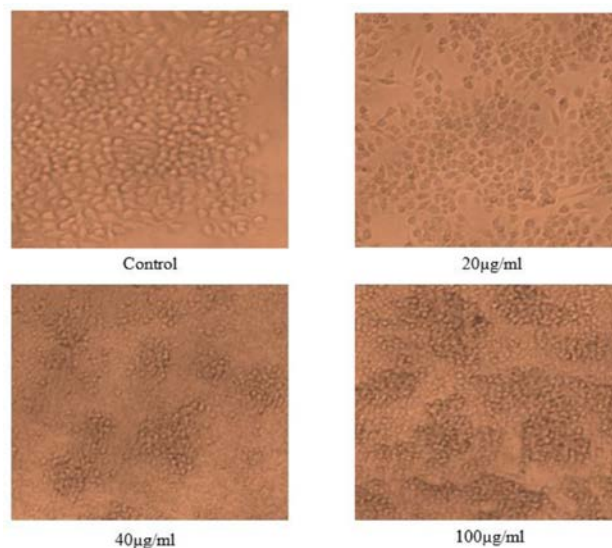
**Figure 1: MTT assay.**

Toxic effect of *Ficus benghalensis* bark extract in Macrophage cells (RAW cells) was studied by MTT assay. The varied concentrations from 10 - 100  $\mu\text{g/ml}$  were showed cytotoxicity effect of *Ficus benghalensis* bark extract in Macrophage cells (RAW 246.7). The  $\text{LC}_{50}$  range of the *Ficus benghalensis* bark extract was found at 50  $\mu\text{g/ml}$  concentration with 51.26% inhibition, whereas inhibition increased in dose dependent manner.



**Figure 2: Trypan Blue exclusion Assay.**

The cell viability of Macrophage cell line (RAW 246.7) treated with *Ficus benghalensis* bark extract was studied by trypan blue assay. The percentage viability of *Ficus benghalensis* bark extract at 10 $\mu\text{g/ml}$  was 86.56% and 48.73% at 50 $\mu\text{g/ml}$  compared to untreated cells. The percentage of cell viability of RAW 264.7 significantly decreased in dose dependent manner.



**Figure 3: Morphometric Analysis.**

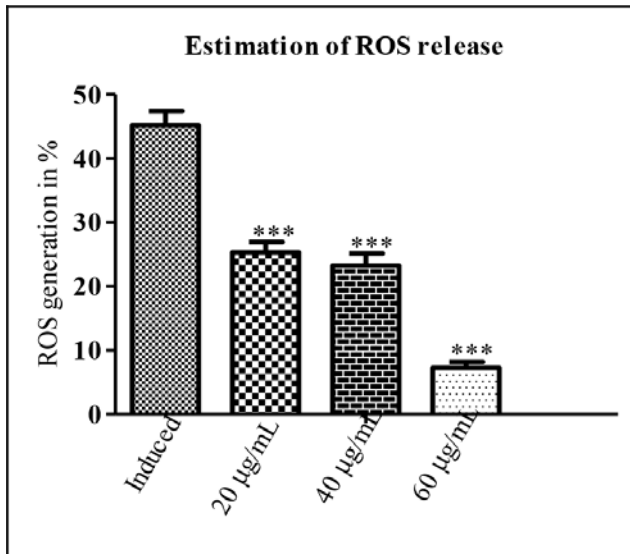
Photomicrograph (20 x) represents morphological changes with detachment, shrinkage, membrane blebbing and disoriented cell shape induced by *Ficus benghalensis* bark extract treatment (0, 20, 40 and 100  $\mu\text{g/ml}$  for 24 hr) when compared with control. The cells in control group showed normal cell morphology and was intact. The images were captured by phase contrast microscope.

### Estimation of ROS Release

LPS elevated ROS levels to  $45.2 \pm 2.17\%$  in the induced groups. However, administering doses of 20, 40, and 60  $\mu\text{g/ml}$  of *F. benghalensis* bark extracts resulted in suppression of ROS to  $25.3 \pm 1.60$ ,  $23.2 \pm 1.91$ , and  $7.28 \pm 0.91\%$  as shown in Figure 4.

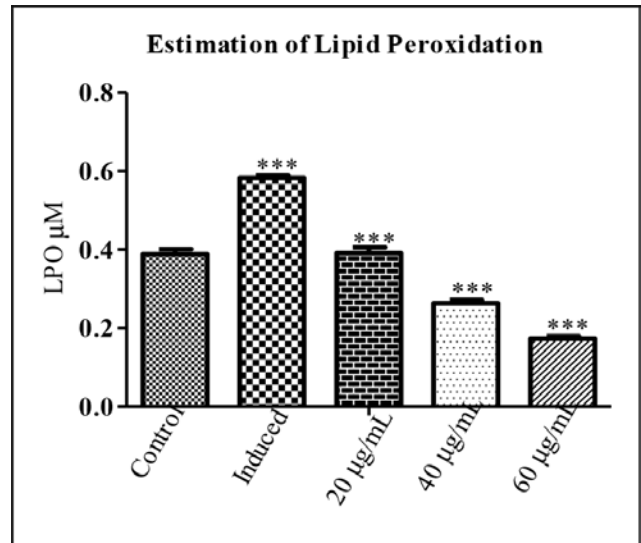
### Nitric Oxide Assay

The nitric oxide levels in the macrophage cells of the LPS-induced group were elevated ( $31.7 \pm 1.20 \mu\text{M/mg}$ ) than that of the control ( $27.6 \pm 1.15 \mu\text{M/mg}$ ). On treating with the bark extract, the elevated levels



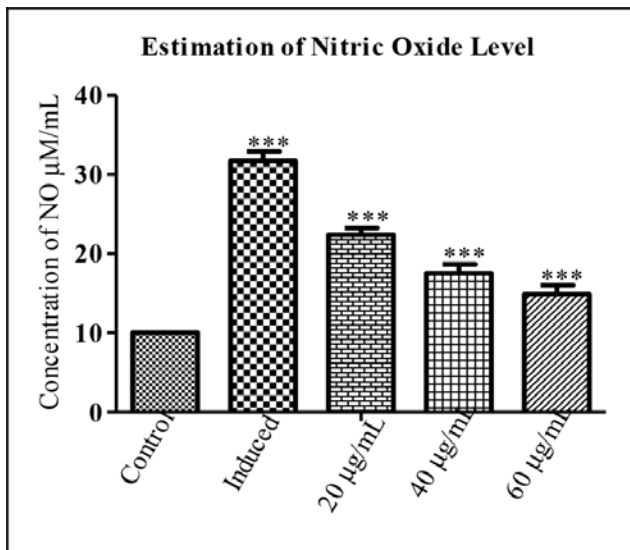
**Figure 4: Estimation of ROS Release.**

Generation of Reactive Oxygen Species between control, induced and treated groups were studied. ROS generation of treated groups was significantly decreased when compared to induced group



**Figure 6: Lipid Peroxidation Assay.**

Lipid peroxidation estimation between control, induced and treated groups were studied. LPO level of treated groups was significantly decreased when compared to induced group



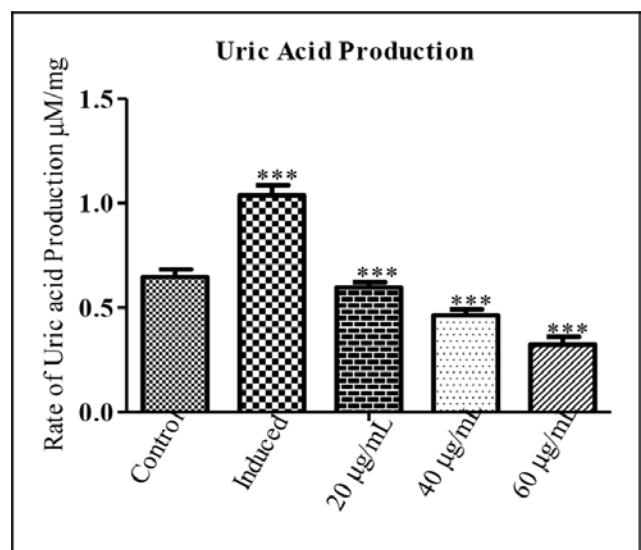
**Figure 5: Nitric Oxide Assay.**

Nitric Oxide level between control, induced and treated groups were studied. NO level of treated groups was significantly decreased when compared to induced group.

were reduced with mean values of  $22.4 \pm 0.83$ ,  $17.5 \pm 1.11$ , and  $14.8 \pm 1.15$  for doses 20, 40, and 60 µg/ml as illustrated in Figure 5.

### Lipid Peroxidation Assay

Compared to the cells in control ( $0.38 \pm 0.01$  µM), the LPO levels in the cells administered with LPS was significantly elevated ( $0.58 \pm 0.006$  µM). On treatment with *Ficus benghalensis* extracts, the cells showed a dose-



**Figure 7: Uric Acid Production.**

Uric acid production between control, induced and treated groups were studied. Uric acid production of treated groups was significantly decreased when compared to induced group

dependent reduction. The LPO levels were  $0.39 \pm 0.01$ ,  $0.26 \pm 0.008$ ,  $0.17 \pm 0.005$  µM for doses 20, 40, and 60 µg/ml (Figure 6).

### Uric Acid Production

The *Ficus benghalensis* extracts caused a significant reduction in the uric acid levels compared to the induced cells. The amount of uric acid in LPS induced

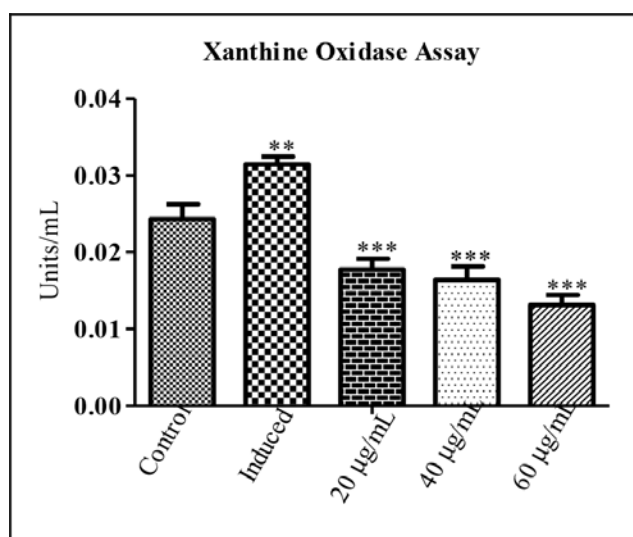
cells was  $1.03 \pm 0.04 \mu\text{M}/\text{mg}$  whereas at different doses such as 20, 40, and 60  $\mu\text{g}/\text{ml}$  resulted in alleviation in the amount of uric acid to  $0.32 \pm 0.03$ ,  $0.46 \pm 0.02$ , and  $0.59 \pm 0.02 \mu\text{M}/\text{mg}$  respectively as shown in Figure 7.

### Xanthine Oxidase Assay

Similar to other assays, xanthine oxidase levels in the LPS-induced cells were higher ( $40.4 \pm 6.50$  units/mL) compared to the normal cells ( $115.02 \pm 5.40$  units/mL). *F. benghalensis* treated cells showed a dose-dependent suppression of XO. At doses 20, 40, and 60  $\mu\text{g}/\text{ml}$  the XO levels were  $18.8 \pm 4.07$ ,  $17.2 \pm 5.22$ , and  $14.4 \pm 5.99$  units/mL as illustrated in Figure 8.

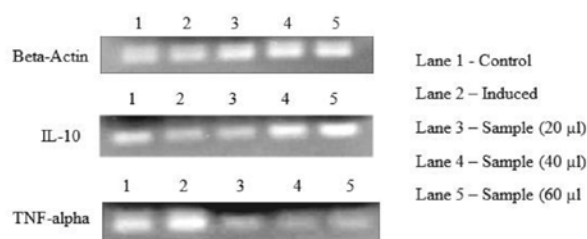
### Gene expression

TNF- $\alpha$  and IL-10 were investigated. Down-regulated gene expression levels were observed for TNF- $\alpha$  and a dose-dependent upregulation in the expression was observed for IL-10 gene as visualized in Figure 9



**Figure 8: Xanthine Oxidase Assay.**

Xanthine Oxidase level between control, induced and treated groups were studied. XO level of treated groups was significantly decreased when compared to induced group



**Figure 9: Gene expression**

Pro-inflammatory and anti-inflammatory cytokines. TNF- $\alpha$  and IL-10 were investigated. Down-regulated gene expression levels were observed for TNF- $\alpha$  and an upregulated expression was observed for IL-10 gene, in a dose-dependent manner.

## DISCUSSION

Over the past few recent years, several studies suggest that hyperuricemia along with inflammation holds a critical role in the etiology and pathogenesis of several metabolic diseases. These necessities the need of identifying a treatment modality that can lower the amount of uric acid and inhibit the inflammatory response. In the current research we indicate the anti-inflammatory and anti-hyperuricemic ability of the *Ficus benghalensis* bark extract using a macrophage cell line RAW 264.7, derived from murine.

Dried bark of *Ficus benghalensis* was extracted with methanol, dried to remove the solvent and used for this study. Methanolic bark extract of *Ficus benghalensis* that has been previously reported for its immunostimulatory effect on fish.<sup>15,24</sup> However, no studies are available on the anti-inflammatory and anti-hyperuricemic activity.

Prior to anti-hyperuricemic and anti-inflammatory studies, viability of RAW 264.7 upon treatment of *Ficus benghalensis* bark extract was investigated using MTT and Trypan Blue exclusion assay. MTT assay also showed a dose dependent limiting in the cell viability with the LC<sub>50</sub> range of the *Ficus benghalensis* bark extract at 50  $\mu\text{g}/\text{ml}$  concentration with 51.26% inhibition. Similar to MTT assay, Trypan blue assay shows 48.73% cell viability at 50  $\mu\text{g}/\text{ml}$  compared to untreated cells.

Bark extracts of *Ficus benghalensis* showed anti-hyperuricemic effects *in vitro* by the ability to suppress xanthine oxidase activity in a dose dependent manner with 60  $\mu\text{g}/\text{ml}$  showing higher inhibition. Besides exhibiting xanthine oxidase inhibitory activity, our results has clearly demonstrated that *Ficus benghalensis* bark extract exerted a dose dependent reduction in the production of uric acid in LPS-induced RAW 264.7 cells. Xanthine oxidase is a vital enzyme in the uric acid biosynthetic pathway and thus by inhibiting its activity the production of uric acid was reduced.<sup>25</sup> Thus, xanthine oxidase is considered as a potent target for preventing uric acid accumulation in hyperuricemia management.<sup>26</sup> The activity of XO was correlated with the uric acid synthesis and ROS leading to the pro-oxidative and pro-inflammatory effects during pathological conditions.<sup>27</sup> High level of uric acid can also be a source to induce oxidative stress. Considering this, we have also estimated the levels of ROS, nitric oxide, lipid peroxidation and inflammatory markers in the LPS induced and *Ficus benghalensis* bark extract treated RAW 264.7 cells.

ROS estimation by NBT reduction assay showed marked increase in the ROS levels at the LPS treated group, whereas in the *Ficus benghalensis* bark extract treated groups showed dose dependent decrease in

the ROS levels with low levels of ROS production at 60 µg/ml group. The observed results was in consistent with the levels of nitric oxide which exhibited the similar trend as ROS. Possible mechanism of anti-inflammatory activity of *Ficus benghalensis* bark extract might be due to ROS production inhibition and providing a radical scavenging effects in oxidative stress. Significant decrease ( $P < 0.0001$ ) in the levels of lipid peroxidation was observed (Figure 6) in *Ficus benghalensis* bark extract treated groups compared with the control and induced groups, indicating that anti-hyperuricemic activity of *Ficus benghalensis* bark extract by inhibiting xanthine oxidase can lead to decreased lipid peroxidation.

Hyperuricemia has been reported to activate NF- $\kappa$ B that lead to the higher expression of pro-inflammatory cytokines.<sup>28</sup> The anti-inflammatory effect studied through gene expression reveals that *Ficus benghalensis* bark extract down regulated the TNF- $\alpha$  expression, and upregulated IL-10 expression. This results indicates the potent anti-inflammatory activity of *Ficus benghalensis* bark extract by downregulating the expression of the pro-inflammatory cytokine TNF- $\alpha$  and upregulation the activity of IL-10, an anti-inflammatory cytokine.

## CONCLUSION

The current study revealed the anti-hyperuricemic and anti-inflammatory activity of *Ficus benghalensis* bark extract in RAW 264.7 cells. The underlying mechanism might be due to the inhibition of xanthine oxidase. Upto the literature studies we have carried out, this study is the first that reports the anti-inflammatory and anti-hyperuricemic effects of *Ficus benghalensis*. These results also provide insights to explore the molecular targets and mechanism of *Ficus benghalensis* bark extract in providing anti-hyperuricemic and anti-inflammatory activity.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **ROS:** Reactive Oxygen Species; **TNF- $\alpha$ :** Tumor Necrosis Factor alpha; **IL- 10:** Interleukin – 10;

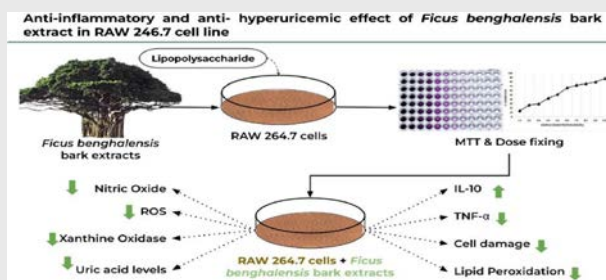
**LPS:** Lipopolysaccharide; **DMSO:** Dimethyl sulfoxide; **DMEM:** Dulbecco's Modified Eagle Medium; **KOH:** Potassium hydroxide; **NED:** N-(1-Naphthyl) ethylenediamine dihydrochloride; **TBARS:** Thiobarbituric acid reactive substances; **H<sub>3</sub>PO<sub>4</sub>:** Phosphoric acid; **MDA:** Malonaldehyde; **AA:** acetic acid; **KCL:** Potassium chloride; **XO:** Xanthine Oxidase; **TCA:** Trichloroacetic acid; **DNA:** Deoxyribo nucleic acid; **PCR:** Polymerase chain reaction; **ANOVA:** Analysis of Variance.

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## PICTORIAL ABSTRACT



## SUMMARY

Excessive uric acid levels in the blood result in inflammation and oxidative stress. This plays a vital role in many diseases. *Ficus benghalensis* bark extract administration in the RAW 246.7 cell line reduced xanthine oxidase, uric acid levels, reactive oxygen species, nitric oxide, and lipid peroxidation. Gene expression studies also showed an increase in IL-10, an anti-inflammatory gene, and a decrease in TNF- $\alpha$ , a pro-inflammatory cytokine. All of these results suggest the Anti-inflammatory and Anti-hyperuricemic Effects of *Ficus benghalensis* Bark Extract.

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