https://doi.org/ 10.33472/AFJBS.6.5.2024. 1659-1679

African Journal of Biological Sciences

Antioxidant, Anti-inflammatory activity of *Tephrosia barberi* J.R. Drumm whole plant in lipopolysaccharide stimulated RAW 264.7 macrophages cell and paw edema induced by carrageenan in wistar rats

P. Kumarnallasivan*[1], S. Sameemabegum[2,]

[*1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Karpagam Academy of Higher education, Coimbatore, India

[2] Research Scholar, Karpagam Academy of Higher education, Coimbatore, India

[*1]Corresponding author: pkns75@gmail.com

Article History
Volume 6, Issue 5, Apr 2024
Received: 01 May 2024
Accepted: 09 May 2024
doi: *10.33472/AFJBS.6.5.2024. 1659-1679*

Abstract

Tephrosia barberi (Fabaceae) J.R. Drumm is a perennial herb that has been used for abdominal pain, fever, peptic ulcer, vatham related diseases, liver disease, and skin disorders.The plan of study is to investigate the antioxidant and anti-inflammatory effects of *Tephrosia barberi* whole plant ethanol extract in lipopolysaccharide- induced RAW 264.7 murine Cells without causing cytotoxicity as well as the effects of carrageenan caused paw edema model in wistar rats. At 10 to 100 µg/mL concentrations ethanolic extract of *Tephrosia barberi* produced dose-reliant radical scavenging property by ABTS and DPPH assay methods. Mean while, inhibitory concentration of (IC_{50}) ethanolic extract of *Tephrosia barberi* showed 29.80µg/mL and 31.83µg/mL by DPPH and ABTS methods, respectively. Pretreatment of RAW 264.7 macropage cells with ethanolic extract of *Tephrosia barberi* produced COX-2 inhibition and LOX inhibition at 48.69µg/mL and 43.50µg/mL (IC_{50}) respectively in anti-inflammatory activity. Whereas, *Tephrosia barberi* extract exhibited significant ($p< 0.005$)inhibition in the production of NO, COX, LOX, and MPO at a higher dose of 100μg/mL, and it was comparable to the reference standard (Diclofenac sodium). Additionally an edema volume produced by the ethanolic extract of *Tephrosia barberi* (400mg/kg) treated rats decreased 2.19-fold which was compared with toxicated rats, and it was statistically typical ($p < 0.05$ and $p < 0.01$) in paw edema caused by carageenan model.At the end of 4 hours of treatment, *Tephrosia barberi* (400mg/kg and 200 mg/kg) showed 58%, 57% edema inhibition, respectively, which was nearly equal to diclofenac (59.2%), a standard drug- treated rat. The results findings confirmed that current research about the ethanolic extract of *Tephrosia barberi's* function as a natural potential anti-inflammatory drug that would be beneficial for inflammatory diseases.

Key words: *Tephrosia barberi*, Anti-oxidant, LPS, Anti-inflammatory, Carageenan

1. Introduction

The development of cancer, diabetes mellitus, cardiovascular disease, neurological diseases, and inflammatory illnesses are the primary causes of oxidative stress. An organism's defensive reaction to an invasion by pathogens, viruses, or parasites is inflammation. When complex enzymes are activated the free radicals are secreted which leading to a number of pro- inflammatory mediators and inflammatory mediators are released throughout the process of acute inflammation [Rahmawati *et al.,* 2021]. Nitric oxide (NO), oxygenated free radicals, lipid mediators and cytokines (TNF, IL-1, IL-6, etc.) are examples of mediators of inflammatory processes. Immune cells, such as macrophages, release these mediators, which have been implicated in several persistent illnesses [Gharred *et al*., 2023]. Regarding these compounds, inflammatory enzymes like inducible Nitric Oxide Synthase and Cyclooxygenase are important during inflammation, which in turn enhances the release of cytokines that promote inflammation namely interleukin-6 and interleukin -1 (Kim *et al.*,2014). Because of these occurrences, macrophages become activated, which in turn causes them to release genes that code for inflammatory agents like COX-2 and iNOS as well as cytokines associated with inflammation [Guha and Macman *et al.*, 2001]. Bacterial lipopolysaccharide, which is an element of gramme-negative bacteria's cell wall, activates macrophages, which then involve them as part of protecting the host and the homeostasis. This process results in the expression of many inflammatory substances, including prostaglandins, cytokines, and NO [Fujithra *et al* 2003; Kang *et al*., 2021]. Fever, pain and swelling are signs and symptoms of an inflammatory response [Jang *et al.,* 2021].

A number of conditions including cancer, rheumatoid arthritis, hepatic damage, diabetes, chronic inflammatory bowel diseases, and asthma have been attributed to inflammatory process [Furst and Zundorf 2014]. There are several treatments available for managing and reducing inflammatory events; examples of these include immunosuppressants, steroids, and nonsteroid anti-inflammatory agents. These medications can have negative side effects. On the other hand, in actual application, our objective is to use the lowest effective dose by the greatest efficacy with the fewest side effects. Therefore, in order to maximize pharmaceutical efficacy and minimize undesirable side effects, we must incorporate natural anti-inflammatory agents into prescription therapy [Ghasemian *et al.,* 2016]. Plants and plant extracts have been used for anti-inflammatory diseases to counteract the synthetic anti-inflammatory drugs which produced more harmful side effects [Furst and Zundorf 2014].

Tephrosia barberi J.R. Drumm is a member of the fabaceae family of perennial herb that has been used for fever, ulcers, dyspnea, liver disorders, skin disorders, and vatham-based diseases [Gamble 1957]. Anti-inflammatory activity in various species of *Tephrosia* has been reported [Obbalareddy *et al.,* 2022; Shenoy *et al.,* 2010]. The previous reports have not yet been reported anti-inflammatory activity of *Tephrosia barberi*. Thus, an effort had made to assess anti-inflammatory efficacy of *Tephrosia barberi* whole plant using both *invivo* and *invitro* models. Based on its anti-inflammatory activity, this herb will be beneficial for rheumatoid arthritis, ulcerative colitis, hepatic damage, diabetics, cancer, stroke, and cardiovascular diseases in the future.

2. Materials and Methods

Source of plant Collection and Preparation of plant extract

In coastal regions Tuticurin, Thirunelveli, and Ramanadhapuram districts, Tamil Nadu, India, the entire plant of *Tephrosia barberi* was collected in February 2021. A voucher specimen (T30072101B/9/2021) was identified by the Central Siddha Research Institute, Arumbakkam, Chennai, and kept in the same for further reference. The entire plant (leaves, stem, flower, fruit, and root) was used for the research study.

The collected *Tephrosia barberi* whole plant was washed with water, pulverised into a coarse powder after being dried in the shade. After defatting, the coarse *Tephrosia barberi* material (250 g) with 1.5 L of petroleum ether, the material was extracted by soxhlet apparatus (continuous hot percolation method) using ethanol as solvent. After the extraction process, the extracts had been collected and evaporate in the open air until it was dry. The dried ethanol extract was kept at 25^{o}C in a refrigerator and used for the following studies.

Anti-oxidant properties of *Tephrosia barberi*

DPPH –Radical scavenging assay

The method used was explained in 1992 by Shimada *et al*. After adding 1.0 mL of DPPH reagent to 4.0 mL of *T. barberi* ethanol extract (10, 20, 40, 80, and 100µg/mL), the absolute amount of DPPH concentration became 0.2 mM. After that, it was quickly mixed and given half an hour to stand. At 517 nm, intensity of absorption had been determined. Vitamin C had been served as basis of standard. A specific formula was used to establish the suppression in DPPH radical scavenging activity

Percentage suppression = $A_O - A_S / A_O \times 100$.

Where, A_O represents control Absorbance and A_S Test sample Absorbance

$ABTS^+$ Radical Scavenging Activity

Giao et al. 2007 described method, ABTS+ decolorization assay had performed with slight modifications (Giao *et al.*, 2007)). An antioxidant capability of plant extract is its ability to scrounge $ABTS^+$ (2, 2'- azino-bis ethyl benzthiazoline - 6 - sulphonic acid) which was produced by mixing potassium persulfate (1/1; v/v) was mixed with a 7 mM ABTS solution and keeping it for 6 hours. To 0.1 mL of varying concentrations of *Tephrosia barberi* extract (0–100 µg/mL), add 0.9 mL ABTS mixture. It was left to stand for fifteen minutes. The ABTS reaction mixture's absorbance has been determined about 734 nm and vitamin C being the typical ingredient. Using following formula, the inhibition was determined:

Percentage Inhibition = A_C- A_S /A_C ×100.

Where, A_C representing control Absorbance, and A_S representing sample Absorbance

***In-vitro* Anti-inflammatory property by LPS stimulated raw cell**

Cell lines and cell culture

The first source of raw 264.7 cells was the National Centre for Cell Sciences (NCCS), located in Pune, India. They were subsequently raised in Dulbecco's Modified Eagles Medium (DMEM), USA, provided by Sigma-Aldrich. The cell line was maintained alive in a tissue culture flask with a volume of 25 cm2 using DMEM which had been improved with L-glutamine, sodium bicarbonate FBS (10%) and penicillin (100 U/ml), streptomycin (100 g/mL), and amphoteracin B (2.5 g/mL) antibiotic solution (Merck, Germany). The cultivated cell lines have been maintained in moistened 5% CO_2 incubator at 37°C.

Experimental procedure

Once the cells have reached 60 percent confluence, 1µL of lipopolysaccharide (LPS: 1µg/mL) was added to activate them. *T. barberi* ethanol extract was tested in different ratios (25, 50 and 100µg/mL) with diclofenac sodium (Reference drug) at different concentrations, which were incorpoated to LPS stimulated raw 264.7cells and incubate for twenty four hours. Following incubation, Cell lysate was utilized in an anti-inflammatory assay (Korambayil et al., 2023).

Cyclooxygenase (COX-2) inhibition

Walker and Gierse (2010) developed the method used to measure the COX activity. Glutathione (5 mM/L), Hemoglobin (5mM/L), and Tris- Hydrochloric acid buffer (pH 8) were added to the Cell lysate (sample: 100µl) and incubate for one min by means of 25°C.

The reaction was initiated by adding 200 mM/L of arachidonic acid. Then, Trichloro acetic acid (10%) in 1N hydrochloric acid (200µL) mixed to end it after being incubated at 37°C for twenty minutes. Following centrifugal process of separation, 1% thiobarbiturate (200µL) should be added. After 20 minutes of boiling, the tubes were chilled. The tubes were centrifuged for three minutes at 2000 rpm after cooling. The absorbance at 632 nm was used to measure cyclooxygenase activity. The cyclooxygenase enzyme % inhibition was computed as follows:

Percentage of cyclo oxygenase inhibition = ((Abs_C – Abst/ Abs_C) × 100

Whereas, Abs_C denotes control Absorbance and Abs_t indicates test sample Absorbance

Lipoxygenase (LOX) inhibition

According to Axelrod et al. (1987), lipoxygenase inhibition had determined. Briefly, process mixture (2 mL final volume) included sodium linoleate (200µL), cell lysate (50µL), and Tris-HCl buffer (pH 7.4). The production of 5-hydroxyeicosatetraenoic acid, a metabolite of arachidonic acid, is reflected by rise in intensity at 234 nm, which denotes LOX inhibition. The enzymatic % inhibition has been calculated using a specific formula.

Percentage of lipooxygenase inhibition = (Control's intensity of absorbance - Sample's intensity of absorbance)/ Control's intensity of absorbance) × 100

Myeloperoxidase (MPO) Inhibition

Myleoperoxidase inhibition was calculated using the technique outlined by Bachoual et al. The test sample mixed in a medium that includes 50 mM 0.57% hexadecyltrimethyl ammonium bromide and potassium phosphate buffer. The amount of MPO was determined from the supernatant of the samples, which were centrifuged over thirty minutes during 4°C at 2000 rpm. The MPO activation of sample had performed by adding 50 mM phosphate buffer (pH 6) with guaiacol (1.67 mg/mL) and water (0.0005%). About 460 nm, an elevation in absorbance was observed. The amount of Myleoperoxidase activation that broken down 1 µM of peroxide per minute at 25°C was one unit of MPO inhibition, which was represented by units per milliliter of sample.

$U = (\Delta OD \cdot 4 \cdot Vt \cdot \text{dilution factor}) / (L \cdot £470 \cdot \Delta t \cdot Vs)$
ΔOD = density change, Vt = total volume (mL) (1.1 mL)
V_S = Absorbance of the sample, Δt = time
L=light path (1 cm), £470 = extinction coefficient for tetraguaiacol (26.6 mM-1·cm-1,)
$U = (\Delta OD \cdot 4 \cdot Vt \cdot \text{dilution factor}) / (L \cdot £470 \cdot \Delta t \cdot Vs)$
ΔOD = density change, Vt = total volume (mL) (1.1 mL)
V_S = Absorbance of the sample, Δt = time
L=light path (1 cm), £470 = extinction coefficient for tetraguaiacol (26.6 mM-1·cm-1,)

Inhibition of inducible Nitric Oxide Synthase

The technique suggested by Salter et al., 1997 has been used for estimating the production of nitric oxide synthase. The cell lysate (test sample) had separated using two milliliters of 4(2-hydroxy ethyl)-1-piperazine ethane sulphonic acid as buffer. The substrate: 0.1 ml enzyme (sample), 0.1 ml -2µmol/L l-arginine, 0.1 ml-10µmol/L (dithiothreitol (DTT) and saturated haemoglobin), 0.1 ml-4µmol/L (Manganese chloride, NADPH, Tetrahydropterin) were all included in the assay system. There was a noticeable increase in absorbance at 401nanometers. Enzyme's % inhibition was computed as follows:

Percentage inhibition = $((Abc - Abs) / Abc) \times 100$

Quantification of Cellular Nitrite

A technique developed by Lepoivre et al. in 1990 has been used to measure amount of nitrite present in Cells. To the cell lysate (0.5mL), 3% sulphosalicylic acid (0.1mL) had added and it was gently shaken for thirty minutes. Following that, it had centrifuged at five thousand revolutions per minute for fifteen mints. The amount of cellular nitrite was estimated using the protein-free supernatant. After adding thirty millilitres of 10% sodium hydroxide with two hundred millilitters supernatant, 300 mL Tris-Hydrochloric acid buffer had been added. The mixture was well combined. Add this to the Griess reagent, kept in dark shade for ten to fifteen minutes. Griess reagent was used as a blank to determine the absorbance at about 540 nm. Meanwhile, Sodium nitrite is the reference.

Statistical analysis

Each experiment had been conducted three times. Findings have shown Mean ± Standard Deviation (SD). Group differences have been assessed by using assessment of variation, or ANOVA, with $p < 0.005$ and $p < 0.01$ being significant.

***Invivo* anti-inflammatory property by carageenan induced paw edema model**

Experimental animal conditions

Both sexes of wistar rats (180-250g) were housed separately in polypropylene enclosures with twelve hours light/dark cycle, at a temperature (23± 2°C) or dew level of 50–60%. Rats have been fed with standard pelleted food, a provided with drinking water at all times. Ethical approval had been obtained from KAHE (Approval no. KAHE/IAEC/2021/11-09/002) Coimbatore, Tamilnadu.

Acute Toxicity Study

An acute toxicological assessment has been carried out in accordance with OECD-423 guidelines, which were acknowledged by CPCSEA, Ministry of Social Justice and Empowerment, Government of India. Albino wistar rats of both sexes were divided into five batches which contained three animals per batch. Rats were administered with ethanolic extract of *Tephrosia barberi* orally (5, 50, 300 and 2000 mg/kg b.w.) and with control (0.5% CMC). Individual animals were examined for the first 30 minutes, then following 24 hours any indications of intoxication and death. Then, it was followed daily and till fourteen days. Using the Abirami et al. methodology, the test dose was established (Abirami *et al.*, 2015).

Design of the study

The animals (female rats) have divided into four groups. Each group comprised six animals. Medication was administered as follows: Ist group (toxicated control): 0.1 mL carrageenan only; IInd group (reference standard): Diclofenac sodium (100 mg/kg) given orally; IIIrd group (low dose of sample): *Tephrosia barberi* (200mg/kg) given orally; IVth group (high dose of sample): *Tephrosia barberi* (400mg/kg) given orally. The sample and standard drugs were dissolved in 0.5% CMC (Saleem *et al.*, 2011).

Study procedure

After an hour, each rat in each group was given by 0.1 mL carrageenan (1%) under the right hind paw's subplantar area. A digital verniar calliper was used to measure the paw volume or paw thickness immediately after carageenan treatment on 0, 1, 2, 3, 4 hrs. Paw thickness increased as a result of the edema. As a result, the paw volume at four hour was determined (Ayertey *et al*., 2021). The proportion of edema suppression in each group in comparison with control group was then calculated as below: (1-Vt/Vc) 100 is the proportion

of inhibition of paw edema. Utilizing Dunnett's test and One-way Annova, the findings were statistically analyzed.

Where, Vt = mean volume of edema formation of test, Vc= volume of edema formation of control

4. Results and Discussion

Anti-oxidant activity of ethanolic extract of *Tephrosia barberi*

Tephrosia barberi ethanol extract was evaluated for its anti-radical properties towards DPPH and ABTS radical cations and compared with standard drug vitamin C. In the current investigation results showed 100µg/mL of plant extract and vitamin C (the reference drug) produced percentage inhibitions of 95.71 and 98.15, respectively, in the DPPH assay method (Figure 1). Meanwhile, the IC_{50} for the ethanolic extract of *Tephrosia barberi* and the standard were calculated using regression analysis by 29.83µg/mL, 22.46µg/mL respectively (Table 1). Furthermore, the observation of plant extract and vitamin C (ascorbic acid) scavenged the ABT radical cation more in the concentration of 100µg/mL and produced percentage reduction of radical cation by 97.71 and 99.86, respectively (Figure 2). Whereas, the IC_{50} of plant extract and standard were calculated using regression analysis and found to be 31.8µg/mL and 14.37µg/mL, respectively, by the ABTS assay method (Table 2). The findings of antioxidant properties of *T. barberi* showed concentration-dependent activity in DPPH and ABTs assays, which leads to good antioxidant properties.

Table 1: Antioxidant activity of *T. barberi* by DPPH assay

Concentration (µg/mL)	**Percentage inhibition**	
	Sample (Plant Extract)	**Standard (VitaminC)**
10	36.23± 0.83	43.47±0.80
20	45.12±0.54	48.14±0.89
40	55.73±0.72	59.16±0.76
80	83.2 ±0.45	87.13±0.56
100	95.71±0.34	98.15±0.71
IC_{50}	29.83 µg/mL	22.46 µg/mL

Value expressed as mean of three reading ± SEM

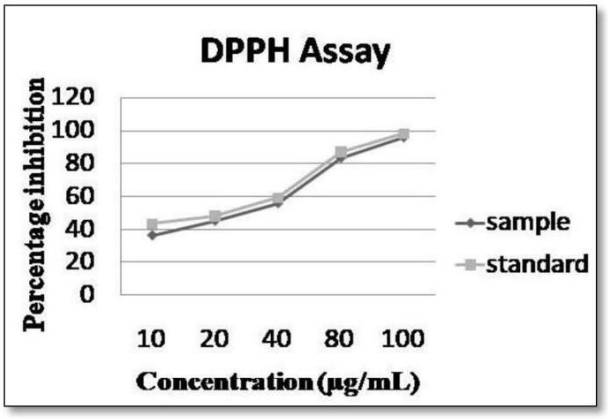

Figure 1: Antioxidant activity of *T. barberi* by DPPH method

Table 2: Antioxidant activity of *T. barberi* by ABTS

Concentration (µg/mL)	Percentage inhibition	
	Sample (Plant Extract)	**Standard (VitaminC)**
10	33.23±0.83	45.42±0.67
20	42.12±0.78	55.15±0.80
40	56.73±0.65	65.12±0.86
80	85.20±0.68	95.15±0.72
100	97.71±0.34	99.86±0.65
IC_{50}	31. 8 µg/mL	14.37 µg/Ml

Value expressed as mean of three reading ± SEM

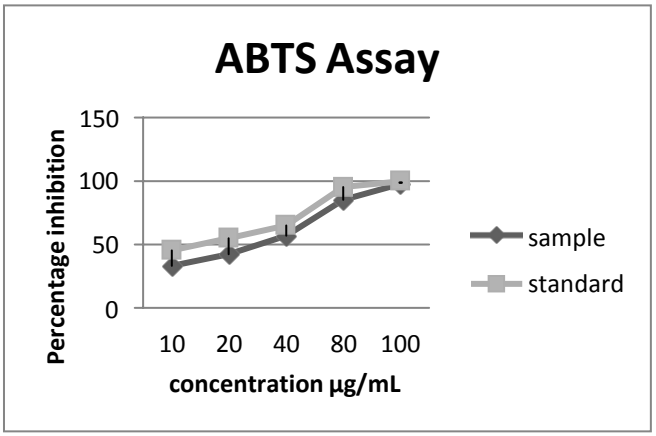

Figure 2: Graphical representation of ABTS+ radical scavenging method

Invitro Anti-inflammatory activity

Effect of *Tephrosia barberi* on Cyclooxygenase (COX) inhibition

The cyclooxygenase enzyme produces prostoglandin, which enhances the action of arachidonic acid in the cells and produces inflammation. The effect of *T.barberi* extract reduced the cyclooxygenase inhibition on lipopolysaccharide stimulated raw cells as dose-related way (Figure 3). Table 3 shows that at a concentration of 100µg/mL, the plant extract

and standard had a stronger inhibition of COX-2 with IC_{50} values 37.46µg/mL and 16.10µg/mL, respectively. According to standard, these findings were significant ($p < 0.005$). Raw 264.7 macropage cells had been used for evaluating anti-inflammatory effects of Tephrosia *barberi* whole plant, which inhibit prostaglandin (proinflammatory mediator) by the enzyme cyclooxygenase. The study result showed *Tephrosia barberi* ethanol extract at high concentrations (100µg/ml) reduced the activity of cyclooxygenase enzymes and reduce inflammation.

Effect of ethanol extract of *Tephrosia barberi* in Lipoxygenase inhibition

Lipoxygenase (LOX), a type of rate-limiting non-heme iron enzyme, mediates the inflammation process. (Wisastara and Dekker 2014). The inhibition of the lipooxygenase enzyme (LPO) can reduce leukotrienes, thereby producing an anti-inflammatory effect. In the current study, ethanol extract of *T. barberi* reduced lipooxygenase inhibition in concentration-dependent ways (Figure 4), leading to a reduction in inflammation, and the IC_{50} values for plant extract and standard drug were determined to be 55.31µg/mL, 27.86µg/mL respectively (Table 4). The ethanolic extract of *T. barberi* 100µg/mL could reduce lipooxygenase by 70.1% and lead to a reduction in inflammation.

Effect of ethanol extract of *Tephrosia barberi* in Myeloperoxidase (MPO) activity

Myleoperoxidase enzymes are released in the extracellular fluid of cells and are directly attributed to production of inflammation. *Tephrosia barberi* inhibits the myleoperoxidase enzyme, which encourages murine macrophages to turn out reactive oxygen species (ROS) and increases secretion of IL-8, TNFα, leads to produce inflammation in macrophages (Gelderman et al., 1998). The results showed that at high concentration, the ethanolic extract of *T. barberi* (100µg/mL) could potentially reduce the myleoperoxidase enzyme activity ($p< 0.01$) compared to LPS (control), leading to a reduction in inflammation (Figure 5 and Table 5).

Table 3: Cyclooxygenase inhibition of *Tephrosia barberi*

Concentration (µg/mL)	**Percentage inhibition**	
LPS	**00**	
	Sample	**standard**
25	35.12±0.34	53.35± 0.08*
50	40.31±0.09	67.82±0.12*
100	63.70±0.12*	90.07±0.23**
IC_{50}	37.46 µg/mL	16.10 µg/mL

Mean ± SEM (n=3) *significant (p < 0.005) sample, standard vs LPS control

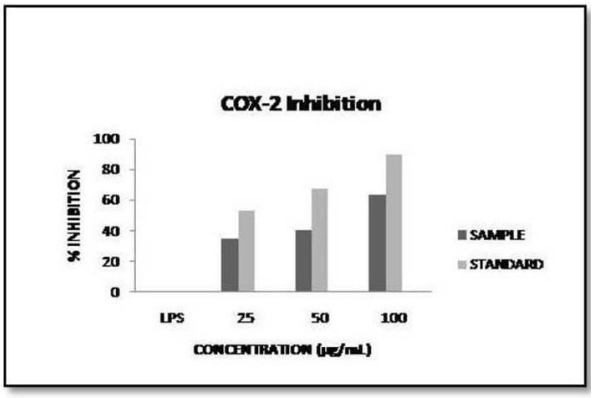

Figure 3: COX-2 inhibition of *T. barberi* ethanol extract

Table 4: Lipoxygenase Inhibition of ethanol extract of *T. barberi*

Concentration (µg/mL)	**Percentage inhibition**	
LPS	**00**	
	Sample	**standard**
25	12.33±0.21	44.11±0.82*
50	35.62±0.10	69.31±1.32*
100	70.14±0.18*	90.13±0.12**
IC_{50}	55.31 µg/mL	27.86 µg/mL

Mean ± SEM (n=3) *significant (p < 0.005) sample, standard vs LPS control

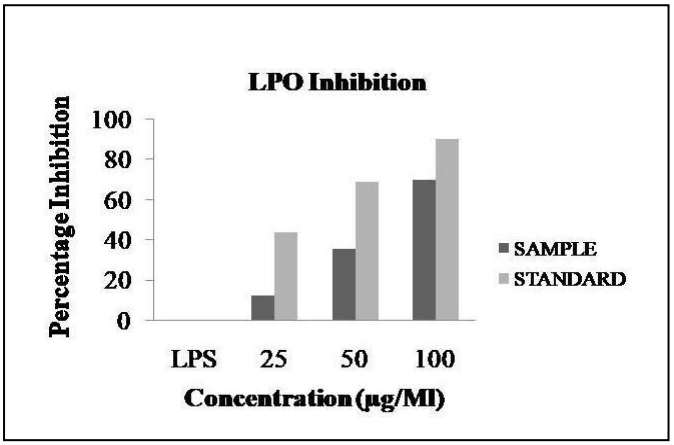

Figure 4: Graphical representation depicting the LOX inhibiton of *T. barberi*

Table 5: Myeloperoxidase enzyme activity of *T.barberi*

Concentration (µg/mL)	Enzyme Activity (U/mL)	
LPS	0.02954±0.001	
	Sample	Standard
25	0.02214± 0.0014	0.00268±0.0008*
50	0.01607±0.0013	0.00156±0.0004*
100	0.00977±0.0006*	0.00087±0.0012**

Mean ± SEM (n=3) *significant (p< 0.01) sample, standard vs LPS control

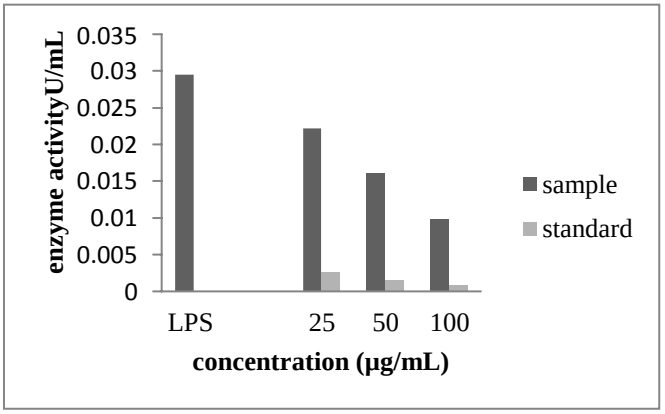

Figure 5: Graphical representation depicting the MPO activity of *T.barberi*

Effect of ethanol extract of *Tephrosia barberi* in Inducible nitric oxide synthase

The amount of Nitric acid production increases cytokines and arachidonic acid levels, which leads to an increased level of inflammation. . *T. barberi's* anti-inflammatory properties

were examined in murine macrophage raw 264.7 cells, which caused significant production of NO over the inflammatory phase. in order to evaluate T. barberi's anti-inflammatory potential, Griess method was used to measure the nitrite concentration in the cell culture medium, which is proportionate to the amount of NO released, Following the addition of *T. barberi* extract, these types of macrophages (RAW 264.7) were induced with LPS intended for duration of 24 hours. The plant extract inhibited mRNA generation of iNOS inhibition in concentration dependent way. The iNOS inhibition of *T. barberi* and standard decreased between 25 and 100 µg/mL in concentrations, which was compared in cells treat with LPS only ($p < 0.01$). The IC_{50} was calculated for plant extract and the standard drug by 80.65µg/mL, 30.44µg/mL, respectively (Table 6 and Figure 6). The inhibitory concentration (IC_{50}) decreased by more than 50%, indicating potential activity in reducing inflammation by plant extract. *Tephrosia barberi* reduced iNOS activity in dose-related way; at 100µg/mL, there was a greater degree of iNOS inhibition, which leads to reduction of inflammation in higher level.

Effect of ethanol extract of *Tephrosia barberi* in Cellular nitrite Levels

The amount of nitrite present in raw cells increases the cytokine level, which leads to inflammation. In the present study, *T. barberi* plant extract with raw cells treated after lipopolysaccharide stimulation decreased the nitrite levels 1.6-, 1.64-, and 2.86-fold at concentration ranges from 25, 50, and 100µg/mL respectively. Meanwhile, the concentrations 25, 50, and 100µg/mL of standard had exhibit decreased nitrite levels 2.65-, 2.14-, and 3.65-fold after LPS stimulation, respectively, which were compared to cells treated with LPS only (Table 7 and Figure 7). The amount of nitrite decreased more at a concentration of 100µg/mL by plant extract and standard, which was significant ($p < 0.005$)) than LPS control and produced potential anti-inflammatory activity.

Table 6: Inducible Nitric Oxide Synthase inhibition of *T. barberi*

Concentration (µg/mL)	Percentage inhibition	
LPS	00	
	Sample	standard
25	12.14±0.23	42.18±0.14*
50	35.12±0.12	68.75±0.35*
100	60.09±0.27*	90.24±0.24**
IC_{50}	80.65 µg/mL	30.44µg/mL

Mean ± SEM (n=3) *significant ($p < 0.01$) Treated control vs LPS control

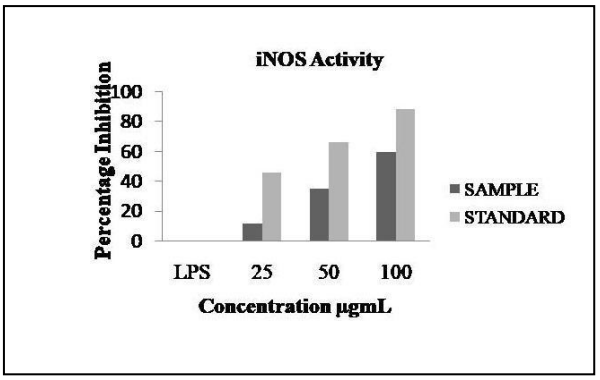

Figure 6: Graphical representation depicting the inducible NOS activity of *T. barberi*

Table 7: Cellular nitrite levels in ethanol extract of *T. barberi*

Concentration (µg/mL)	Concentration of Nitrite (µg)	
LPS	830.61±0.001	
	Sample	Standard
25	777.645±0.08	312.684±0.07
50	594.495±0.06	387.459±0.08
100	289.555±0.12*	227.465±0.01*

Mean ± SEM (n=3) *significant ($p < 0.005$) sample, standard vs LPS control

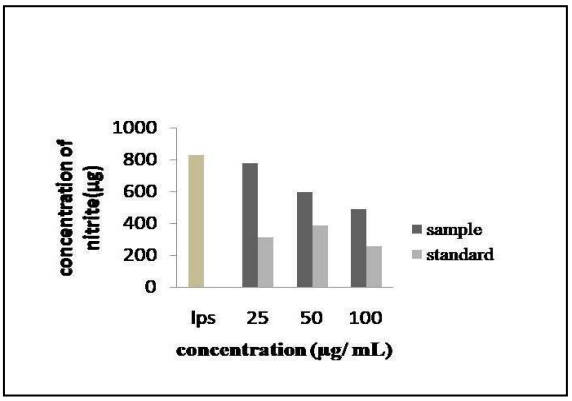

Figure 7: Graphical representation depicts cellular nitrite activity of *T. barberi*

***Invivo* anti-inflammatory activity**

Acute Toxicity Studies

Up to 2000mg/kg *Tephrosia barberi* extract had been administered orally to the rats as part of the acute toxicity trials, but there was no harm, no death, and no observable behavioral patterns. It is the cutoff dose, and 200 mg/kg ((1/10th dose), 400 mg/kg (1/5th dose) were chosen as efficient doses for further research in accordance with toxicity recommendations.

Effectiveness of *T. barberi* on Carageenan induced model of paw edema

The carageenan-induced paw edema model for anti-inflammatory, reduction of edema happened in two phases. Proinflammatory markers like histamine, serotonin, and bradykinin were released during the early phase (0–1 hr) and are responsible for the production of edema. In the late phase (3–4 hr), prostaglandins and other cytokines such as IL-1, IL-6, IL-10, and TNF-α are crucial for inflammation. (Dzoyem *et al.*, 2017). We observed all the tested groups showed edema reduction was higher in the late phase due to inhibition of cytokines. The ethanolic extract of *Tephrosia barberi* at 200 mg/kg reduced the volume of edema in the early phase, which was higher than standard, whereas in the late phase it had decreased. Oral administration of an ethanol extract of *Tephrosia barberi* dose-dependently reduced carrageenan-induced paw edema. In the present study, the volume of edema produced by the ethanolic extract of *Tephrosia barberi* (400mg/kg)-treated rat decreased 2.19-fold after 4hr treatment which was compared with toxicated rats, and it was statistically remarkable ($p < 0.05$ and 0.01). In same way, rats given by 200mg/kg ethanolic extract of *Tephrosia barberi* decreased the mean volume of edema by 2.06-fold after 4hr of treatment. At the end of 4 hours of treatment, *Tephrosia barberi* (400 mg/kg, 200 mg/kg) showed 58%, 57% edema inhibition, respectively which was nearly equal to diclofenac (59.2%), a standard drug treated rats (Table 8)

Table 8: Measurement of edema reduction by *Tephrosia barberi*

Group	Mean volume of edema (mm) at different time interval					% decrease in paw volume at 4h
	0h	1 h	2 h	3h	4h	
Negative control	3.09±0.019	3.12±0.027	3.14±0.031	3.16±0.056	3.18±0.042	-
Standard (diclofenacsodium)	2.66±0.023	2.61±0.094*	2.11±0.052*	1.75±0.017**	1.39±0.095**	59.2
TestI(200mg/kg)	2.37±0.015	2.22±0.028*	2.02±0.067*	1.81±0.235**	1.54±0.412**	57
TestII(400mg/kg)	2.86±0.036	2.59±0.037*	2.26±0.043*	1.78±0.078**	1.45±0.145**	58

The data are shown as mean ± SEM (n = 6), with p*<0.05 and p **<0.01 significant differences from the toxicated control group. The Annova and Dunnets tests were used to analyze the data.

. 4. Conclusion

In the present work, *invitro* antioxidant, anti-inflammatory using raw cells by cyclooxygenase inhibition, lipooxygenase inhibition, myleoperoxidase inhibition, inducible nitric oxide synthase, cellular nitrite expressions and *invivo* by experimentally induced paw edema in a wistar rat's model using an ethanolic extract of *Tephrosia barberi* all have been evaluated. LPS-induced production of nitrite, LOX, COX-2, iNOS, were successfully reduced by pretreatment with ethanolic extract of *Tephrosia barberi* in raw (264.7) macrophage cell. All the inflammatory expressions were higher at a concentration of 100µg/mL. Furthermore, orally administered ethanol extract of *Tephrosia barberi* reduced paw edema by dose and time-related way in a model of carageenan induced paw edema. Hence, the findings of results showed ethanolic extract of T*ephrosia barberi* possessed potential anti-inflammatory activity. The good antioxidant property of *Tephrosia barberi* also attributed to anti-inflammatory activity. Hence, the present research concluded that *Tephrosia barberi* J.R. Drumm is a natural, promising potential anti-inflammatory drug that would be useful for inflammatory diseases.

References

1. **Abirami, A., Nagarani, G., and Siddhuraju, P., (2015).** Hepatoprotective effect of leaf extracts from *citrus hystrix* and *c.maxima* against paracetamol induced liver injury in rats. Food Science and Human Wellness, 4(1), pp. 35-41. https://doi.org/10.1016/j.fshw.2015.02.002.
2. **Axelrod, B., Cheesbrough, T. M., and Laakso, S., (1981).** Lipoxygenases in soybean. : EC 1.13.11.12 Linoleate: Oxygen oxidoreductase. Meth. Enzymology, 71, pp. 441-451. https://doi.org/10.1016/0076-6879(81)71055-3.
3. **Bachoual, R., Talmoudi, W., Boussetta, T., Braut, F., and El-Benna, J., (2011).** An aqueous pomegranate peel extract inhibits neutrophil myeloperoxidase *in vitro* attenuate lung inflammation in mice, Food and ChemicalToxicology,49(6),pp.1224-1228. https://doi.org/10.1016/j.fct.2011.02.024
4. **Dzoyem, J.P, McGaw, L.J., Kuete, U. V., Bakowsky, U., (2017).** Text book of Medicinal Spices and Vegetables from Africa, pp. 239-270.
5. **Gharred, N., Ali, L.M.A., Bettache, N., Dridi-Dhaouadi, S., Morere, A., and Menut, C., (2023).** *In Vitro* Anti-inflammatory Activity of Three *Inula* Species Essential Oils in Lipopolysaccharide-Stimulated RAW 264.7 Macrophages. Chemistry Africa, 6, pp. 1933–1942. DOI:10.1007/s42250-023-00641-3
6. **Giao, M.S., Gonzalez, S.M.L., Rivero, P.M.D., Pereira, C.I., Pintado, M.E., and Malcata, F.X., (2007).** Infusions of Portuguese medicinal plants: Dependence of final antioxidant capacity and phenolic content on extraction features. J Science Food Agriculture, 87(14), pp.2638-2647. https://doi.org/10.1002/jsfa.3023.
7. **Guchu, B.M., Machocho, A.K., Mwihia, S.K., and Ngugi M.P., (2020)**. *In Vitro* Antioxidant activities of methanolic extracts of *Caesalpinia volkensii* O. Hoffm., and Acacia hockii De Wild, Evidence-Based Complementary and Alternative Medicine, pp. 2020. https://doi.org/10.1155/2020/3586268
8. **Fujithra, M., Murol, M., Tanomoto, K., Suzuki, T., and Ikeda, A. H., (2003).** Molecular mechanisms of macropage activation and deactivation by lipopolysaccharide; role of the receptor complex, Pharmacol and Therapeutics, 100(2), pp. 171-184. https://doi.org/10.1016/j.pharmthera.2003.08.003.

9. **Furst, R., and Zundorf, I., (2014).** Plant-Derived Anti-Inflammatory Compounds: Hopes and Disappointments regarding the Translation of Preclinical Knowledge into Clinical Progress, Herbal Medicines for InflammatoryDiseases, 2014,pp.1-9. https://doi.org/10.1155/2014/146832.
10. **Gamble, J.S., (1957).** Text book of Flora of presidency of Madras 1, pp. 220.
11. **Ghasemian, M., Owlia, S., and Owlia, M.B., (2016).** Review of anti-inflammatory herbal medicines. Advances in Pharmacological and Pharmaceutical Science 2016(1), pp. 1-11. https://doi.org/10.1155/2016/9130979
12. **Guha, M, and Mackman, N., (2001).** LPS induction of gene in human monocytes. Cellular Signaling, 13(2), pp. 85-94. https://doi.org/10.1016/S0898-6568(00)00149-2
13. **Jang, G., Lee S., Hong J., Park B., Kim D., and Kim C., (2021)**. Anti-inflammatory effect of 4,5-dicaffeoylquinic acid on RAW264.7 cells and a rat model of inflammation. Nutrients, 13(10), pp. 3537. https://doi.org/10.3390/nu13103537
14. **Kang, J., Chung, Y., and Chang-Gu Hyun C., (2021).** Anti-Inflammatory Effects of 6 methylcoumarin in LPS-Stimulated RAW 264.7 macrophages via regulation of MAPK and NF-κB signaling pathways**,** Molecules, 26(17), pp. 5351. https://doi.org/10.3390/molecules26175351
15. **Kedare, S.B., and Singh, R.P., (2011)**. Genesis and development of DPPH method of antioxidant assay, J Food Sci Technol., 48(4), pp. 412–422. https://doi.org/10.1007/s13197-011-0251-1.
16. **Kim, H, and Kim, J., (2014).** A guide to genome engineering with programmable nucleases. Nat Revision of Genetics, 15(5), pp.321-34. https://doi.org/10.1038/nrg3686.
17. **Korambayi, P.M., Varkey, P., and Tharakan, M., (2023).** Anti-inflammatory and cytotoxicity assay of Cardamom-based nutraceuticals in comparison with 1,8 –Cineol. Journal of Drug Delivery & Therapeutics, 13(10), 88-93. https://doi.org/10.22270/jddt.v13i10.5978.
18. **Lepoivre, M., Chenais, B., Yapo, A., Lemaire, G., Thelander, L., Tenu, J.P., (1990).** Alterations of ribonucleotidereductase activity following

induction of the nitrite-generating pathway in adenocarcinoma cells. J Bio Chem, 265(24), pp. 14143-14152. DOI:10.1016/S0021-9258(18)77279-7.

19. **Obbalareddy, S., Kolli, P.K,, Yejella, R.P., Athili, L.D and Ponnada, S. (2022).** A review on *Tephrosia* genus. International Journal of Research in Pharmacy and Chemistry, 12(3), pp. 208-247.
20. **Rahmawati, L., Park, S.H., Kim, D.S., Lee, H.P,, Naziz, N., Lee, C.Y., Kim, S.A., Jang, S.G., Kim, D.S., and Cho, J.Y., (2021).** Anti-inflammatory activities of the ethanol extract of *Prasiola japonica*, an edible freshwater green algae, and its various solvent fractions in LPS-induced macrophages and carrageenan-induced paw edema via a AP-1 pathway, Molecules, 27(1):194. https://doi.org/10.3390/molecules27010194.
21. **Saleem, T.K.M., Azeem, A.K., Dilip, C., Sankar, C., Prasanth, N.V., and Duraisami, R., (2011)**. Anti-inflammatory activity of the leaf extracts of *Gendarussa vulgaris* Nee. 1(2), pp. 147–149. https://doi.org/10.1016/S2221-1691(11)60014-2.
22. **Salter, M., Duffy C., Garthw Saite, J., and Strijbos, P.J., (1996).** Ex *vivo* measurement of brain tissue nitrite and nitrate accurately reflects nitric oxide synthase activity i*nvivo*, J. Neurochem, 66 (4), pp. 1683–1690. https://doi.org/10.1046/j.1471-4159.1996.66041683.
23. **Shenoy, S., Shwetha, K., Prabhu, K., Maradi, R., Bairy, K.L., Shanbhag, T., (2020).** Evaluation of antiinflammatory activity of *Tephrosia purpurea* in rats, Asian Pacific Journal of Tropical Medicine, 3(3), pp. 193-195. https://doi.org/10.1016/S1995-7645(10)60007-7.
24. **Shimada, K., Fujikawa, K., Yahara, K and Nakamura, T., (1992).** Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chemistry, 40, pp. 945- 953. https://doi.org/10.1021/jf00018a005.
25. **Sneha, S., Shwetha, K., Prabhu, K., Maradi, R., Bairy, K.L., and Shanbhag, T., (2010).** Evaluation of antiinflammatory activity of *Tephrosia purpurea* in rats. Asian Pacific Journal of Tropical Medicine 2010; 193-195. https://doi.org/10.1016/S1995-7645(10)60007-7.

26. **Vinegar, R., Schreiber, W., and Hugo R., (1969).** Biphasic development of carrageenin edema in rats. Journal of Pharmacology and Experimental Therapeutics, 166 (1), pp. 96-103.
27. **Wisastra, R., and Dekker, F.J., (2014)**. Inflammation, cancer and oxidative Lipoxygenase activity are Intimately Linked. Cancers (Basel), 2014; 6(3): 1500–1521. https://doi.org/10.3390/cancers6031500.
28. **Walker, M.C., and Gierse, J.K., (2010).** *In Vitro* Assays for Cyclooxygenase Activity and Inhibitor Characterization. In: Ayoub, S.S, Flower, R., Seed, M. (eds) Cyclooxygenases. Methods in Molecular Biology, 644. Humana Press. https://doi.org/10.1007/978-1-59745-364-6_11.