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EXPERIMENTAL ASSESSMENT OF THE ANTI-ARTHRITIC POTENTIAL OF TEPHROSIA PURPUREA L.: A COMPREHENSIVE PHARMACOLOGICAL INVESTIGATION

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Abstract

Rheumatoid arthritis is a chronic autoimmune disorder marked by progressive joint destruction and systemic inflammation. Current therapies are often limited by side effects and inconsistent efficacy, prompting interest in natural alternatives. Tephrosia purpurea L. (Sharapunkha), traditionally used in Ayurveda for inflammatory ailments, has shown promise against rheumatism and joint disorders. This study evaluated the anti-arthritic potential of hydro-alcoholic extract of T. purpurea leaves in Freund's complete adjuvant (FCA)-induced arthritis in Wistar rats. It further assessed inflammatory biomarkers, safety through acute toxicity studies, and possible mechanisms of action. Acute toxicity was assessed following OECD 423 guidelines. Arthritis was induced in 36 male Wistar rats using FCA. Animals were orally treated with T. purpurea extract (200 and 400 mg/kg) for 21 days. Paw edema, arthritic score, inflammatory cytokines, biochemical indices, and histopathology were analyzed. The extract was safe (LD_{50} >2000 mg/kg) and significantly reduced paw swelling, arthritic scores, and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6). Histology confirmed reduced synovial inflammation and cartilage protection. The findings highlight T. purpurea as a promising natural therapeutic for rheumatoid arthritis.

Keywords: *Tephrosia purpurea[1], rheumatoid arthritis[2], anti-inflammatory[3], cytokines[4], Freund's adjuvant[5].*

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease affecting approximately 1% of the global population, characterized by persistent inflammation of synovial joints leading to cartilage destruction, bone erosion, and eventual joint deformity (Rao et al., 2020). The pathogenesis involves complex interactions between genetic predisposition, environmental factors, and immune dysregulation, resulting in the activation of inflammatory cascades mediated by pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) (Comalada et al., 2005). Current therapeutic strategies for RA management include disease-modifying antirheumatic drugs (DMARDs), biological agents, and corticosteroids. However, these treatments are associated with significant adverse effects including immunosuppression, hepatotoxicity, and increased infection risk, highlighting the urgent need for safer alternative therapies (Nair et al., 2007). Natural products derived from medicinal plants have gained considerable attention due to their multi-target therapeutic approach and relatively favorable safety profiles (Singh et al., 2014).

Tephrosia purpurea (L.) Pers., belonging to the family Fabaceae, is a perennial herb widely distributed throughout the Indian subcontinent and traditionally known as "Sharapunkha" or "Sarwa wranvishapaka" in Ayurvedic medicine (Palbag et al., 2014). The plant has been extensively used in traditional medicine systems for treating various inflammatory conditions including rheumatism, hepatic disorders, spleen enlargement, and wound healing (Akanksha et al., 2014). Phytochemical investigations have revealed the presence of numerous bioactive compounds including flavonoids (rutin, quercetin), isoflavonoids (tephrosin, pongamol), triterpenes (lupeol), and steroids (β-sitosterol) which collectively contribute to its therapeutic properties (Chang et al., 2000). Recent pharmacological studies have demonstrated various biological activities of *T. purpurea* including anti-inflammatory, antioxidant, hepatoprotective, antimicrobial, and immunomodulatory effects (Gokhale & Saraf, 2000). The anti-inflammatory properties are attributed to the inhibition of pro-inflammatory mediators and modulation of immune responses, suggesting potential therapeutic applications in inflammatory arthritis (Jain et al., 2009). However, comprehensive evaluation of its anti-arthritic potential using standardized experimental models remains limited, necessitating systematic investigation to establish scientific validity for its traditional use in rheumatic disorders.

2. Literature Review

Traditional and Ethnomedicinal Uses

Tephrosia purpurea has been extensively documented in classical Ayurvedic texts and traditional medicine systems across various cultures. The plant is particularly valued for its wound healing properties, earning the Sanskrit name "Sarwa wranvishapaka," which translates to "healer of all wounds" (Rao et al., 2020). Traditional practitioners have utilized different parts of the plant, including leaves, roots, and whole plant extracts, for treating diverse inflammatory conditions such as rheumatism, arthritis, hepatomegaly, splenomegaly, and various skin disorders (Palbag et al., 2014).

Phytochemical Profile

Extensive phytochemical investigations have identified numerous bioactive compounds in *T. purpurea*, including flavonoids (rutin, quercetin, kaempferol), isoflavonoids (tephrosin, pongamol, semiglabrin), chalcones (tephropurpurin), triterpenes (lupeol, oleanolic acid), and steroids (β-sitosterol, stigmasterol) (Ahmad et al., 1999). These compounds are recognized for their diverse pharmacological properties, particularly anti-inflammatory and immunomodulatory activities. Rutin and quercetin, in particular, have been extensively studied for their ability to inhibit inflammatory cytokines and modulate immune responses (Edwards et al., 2007).

Anti-inflammatory and Related Activities

Previous studies have demonstrated significant anti-inflammatory activity of *T. purpurea* extracts in various experimental models. The flavonoidal fraction has shown immunomodulatory effects by inhibiting delayed-type hypersensitivity reactions and reducing inflammatory mediator production (Gokhale et al., 2003). The extract has also exhibited antioxidant properties, reducing oxidative stress markers and protecting against lipid peroxidation (Nile & Park, 2014). These findings provide scientific support for the traditional use of the plant in inflammatory conditions and suggest potential therapeutic applications in rheumatoid arthritis.

Safety and Toxicological Profile

Toxicological evaluations have consistently demonstrated the safety of *T. purpurea* extracts, with oral LD_{50} values exceeding 2000 mg/kg in rodent models (Khatri et al., 2009). Subacute toxicity studies have shown no significant adverse effects at therapeutic doses, indicating a favorable safety profile suitable for long-term

therapeutic use (Gora et al., 2014). This safety profile, combined with traditional use evidence, supports the potential development of *T. purpurea* as a therapeutic agent for chronic inflammatory conditions.

3. Objectives

The present study was designed with the following specific objectives:

1. To evaluate the acute toxicity profile of hydro-alcoholic extract of *Tephrosia purpurea* leaves in accordance with OECD guidelines 423 to establish safety parameters
2. To investigate the anti-arthritic potential of *T. purpurea* extract using Freund's complete adjuvant-induced arthritis model in Wistar rats
3. To assess the effects on inflammatory biomarkers including pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and biochemical parameters
4. To examine histopathological changes in joint tissues and evaluate the protective effects of the extract against arthritic damage

4. Methodology

Plant Material and Extract Preparation

Fresh leaves of *Tephrosia purpurea* were collected from the medicinal plant garden of the Department of Pharmacognosy during the month of October 2023. The plant was authenticated by Dr. K.S. Laddha, Department of Pharmacognosy, University Department of Chemical Technology, Mumbai, India (Voucher specimen no. TP-2023-15). The leaves were washed, shade-dried, and powdered using a mechanical grinder. Hydro-alcoholic extract was prepared by macerating 500g of powder in 70% ethanol (1:10 ratio) for 72 hours with intermittent shaking. The extract was filtered, concentrated under reduced pressure at 40°C, and dried to obtain a brownish residue with a yield of 12.8% w/w.

Phytochemical Screening

Preliminary phytochemical screening was performed using standard qualitative tests to identify the presence of major secondary metabolites including alkaloids, glycosides, flavonoids, tannins, terpenoids, and steroids. Total phenolic and flavonoid content were determined spectrophotometrically using Folin-Ciocalteu reagent and aluminum chloride colorimetric methods, respectively.

Experimental Animals

Male Wistar rats (150-200g) were obtained from the National Institute of Nutrition, Hyderabad, and housed in standard laboratory conditions (12:12 hour light-dark cycle, 25±2°C, 55±5% humidity) with free access to standard pellet diet and water. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC/2023/PHARM/08) in accordance with CPCSEA guidelines.

Acute Toxicity Study

Acute oral toxicity was evaluated following OECD guideline 423 (Acute Toxic Class Method). Female Wistar rats were randomly divided into control and test groups (n=3 each). After overnight fasting, animals received single oral doses of *T. purpurea* extract at 300 and 2000 mg/kg body weight. Animals were observed continuously for the first 4 hours and then periodically for 14 days for signs of toxicity, behavioral changes, and mortality. The LD_{50} was calculated using the AOT software program.

Freund's Complete Adjuvant-Induced Arthritis

Arthritis was induced according to the method described by Winter et al. (1962) with modifications. Male Wistar rats (n=36) were randomly divided into six groups of six animals each: Group I (Normal control), Group II (Arthritic control), Group III (Standard - Diclofenac sodium 10 mg/kg), Group IV (*T. purpurea* 100 mg/kg), Group V (*T. purpurea* 200 mg/kg), and Group VI (*T. purpurea* 400 mg/kg). Arthritis was induced by single subcutaneous injection of 0.1 mL Freund's complete adjuvant (1 mg/mL heat-killed *Mycobacterium tuberculosis* in liquid paraffin) into the right hind paw. Treatment was initiated on day 0 and continued for 21 days.

Assessment Parameters

- **Paw Volume Measurement:** Paw volume was measured using a digital plethysmometer (Ugo Basile, Italy) on days 0, 3, 7, 14, and 21. The difference between injected and non-injected paw volumes was calculated as an index of inflammation.
- **Arthritic Score:** Clinical arthritis was evaluated using a 0-4 scoring system: 0 = normal, 1 = slight swelling and erythema, 2 = low-to-moderate edema, 3 = pronounced edema with limited joint usage, 4 = excessive edema with joint rigidity.
- **Body Weight:** Animals were weighed weekly throughout the study period to assess the impact of arthritis and treatment on general health.

Biochemical Analysis

Blood samples were collected on day 21 by cardiac puncture under anesthesia. Serum was separated and analyzed for inflammatory cytokines (TNF-α, IL-1β, IL-6) using ELISA kits (R&D Systems, USA), C-reactive protein (CRP), rheumatoid factor (RF), and liver function parameters (AST, ALT, ALP) using standard clinical chemistry methods.

Histopathological Examination

On day 21, animals were sacrificed, and ankle joints were carefully dissected, fixed in 10% neutral buffered formalin, decalcified in 10% EDTA solution, processed, and embedded in paraffin. Sections (5µm) were stained with hematoxylin and eosin and examined microscopically for inflammatory changes, synovial hyperplasia, cartilage damage, and bone erosion.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed using GraphPad Prism 8.0 software. One-way ANOVA followed by Tukey's post-hoc test was employed for multiple group comparisons. P-values <0.05 were considered statistically significant.

5. Results

Phytochemical Analysis and Extract Yield

The hydro-alcoholic extract of *T. purpurea* leaves yielded 12.8% w/w of dark brown residue. Phytochemical screening revealed the presence of flavonoids, tannins, glycosides, terpenoids, and steroids. Total phenolic content was determined to be 18.44 ± 0.13% w/w gallic acid equivalent, while total flavonoid content was 2.54 ± 0.12% w/w quercetin equivalent, indicating substantial antioxidant potential.

Acute Toxicity Study

The acute toxicity study revealed no mortality or signs of toxicity at the tested doses (300 and 2000 mg/kg). All animals remained normal throughout the 14-day observation period with no significant changes in behavior, food consumption, or body weight. The LD_{50} was estimated to be greater than 2000 mg/kg, classifying the extract as practically non-toxic according to OECD classification.

Effect on Paw Volume

Table 1: Effect of *T. purpurea* extract on paw volume in adjuvant-induced arthritis

Treatment Groups	Day 0	Day 3	Day 7	Day 14	Day 21
Normal Control	0.28±0.02	0.29±0.02	0.30±0.02	0.31±0.02	0.32±0.02
Arthritic Control	0.29±0.02	1.45±0.08	2.18±0.12	2.89±0.15	3.24±0.18
Diclofenac (10 mg/kg)	0.30±0.02	0.98±0.06***	1.34±0.09***	1.67±0.11***	1.89±0.13***
TP 100 mg/kg	0.28±0.02	1.32±0.07	1.89±0.11*	2.34±0.14*	2.67±0.16*
TP 200 mg/kg	0.29±0.02	1.18±0.06**	1.65±0.10**	2.01±0.12**	2.31±0.14**
TP 400 mg/kg	0.30±0.02	1.02±0.06***	1.42±0.09***	1.75±0.11***	1.98±0.12***

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 compared to arthritic control.

The paw volume measurements demonstrate a significant dose-dependent reduction in inflammation with *T. purpurea* treatment. On day 21, the arthritic control group showed maximum swelling (3.24±0.18 mL) compared to normal control (0.32±0.02 mL). Treatment with *T. purpurea* at 400 mg/kg significantly reduced paw volume to 1.98±0.12 mL (P<0.001), showing comparable efficacy to diclofenac sodium (1.89±0.13 mL). The 200 mg/kg dose also demonstrated significant anti-inflammatory activity (2.31±0.14 mL, P<0.01), while the 100 mg/kg dose showed moderate but significant effects (2.67±0.16 mL, P<0.05). These results indicate that *T. purpurea* extract possesses potent dose-dependent anti-inflammatory properties in the adjuvant-induced arthritis model.

Arthritic Score Assessment

Table 2: Effect of *T. purpurea* extract on arthritic score

Treatment Groups	Day 7	Day 14	Day 21
Normal Control	0.00±0.00	0.00±0.00	0.00±0.00
Arthritic Control	2.83±0.17	3.50±0.22	3.83±0.17
Diclofenac (10 mg/kg)	1.67±0.21***	2.00±0.26***	2.17±0.31***
TP 100 mg/kg	2.33±0.21*	2.83±0.17*	3.17±0.17*
TP 200 mg/kg	2.00±0.26**	2.33±0.21**	2.67±0.21**
TP 400 mg/kg	1.67±0.21***	1.83±0.17***	2.00±0.26***

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 compared to arthritic control.

The arthritic score evaluation demonstrates progressive improvement with *T. purpurea* treatment across all time points. By day 21, the arthritic control group exhibited severe arthritis with a score of 3.83±0.17, while the highest dose of *T. purpurea* (400 mg/kg) significantly reduced this to 2.00±0.26 (P<0.001), comparable to diclofenac treatment (2.17±0.31). The 200 mg/kg dose also showed significant improvement (2.67±0.21, P<0.01), indicating substantial clinical benefit. The progressive reduction in arthritic scores from day 7 to day 21 suggests sustained therapeutic efficacy. The dose-response relationship is clearly evident, with higher doses providing greater protection against arthritic progression.

Inflammatory Cytokine Analysis

Table 3: Effect of *T. purpurea* extract on serum inflammatory cytokines

Treatment Groups	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
Normal Control	45.2±3.1	28.4±2.3	12.8±1.1
Arthritic Control	298.7±18.6	187.3±12.4	142.6±9.8
Diclofenac (10 mg/kg)	128.4±9.2***	89.7±7.1***	58.3±4.6***
TP 100 mg/kg	234.1±15.7*	156.2±10.8*	118.4±8.2*
TP 200 mg/kg	187.6±13.4**	124.8±9.3**	89.7±6.8**
TP 400 mg/kg	142.3±10.9***	98.4±7.9***	64.2±5.1***

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 compared to arthritic control.

The cytokine analysis reveals significant modulation of inflammatory mediators by *T. purpurea* extract. TNF-α levels were markedly elevated in the arthritic control group (298.7±18.6 pg/mL) compared to normal controls (45.2±3.1 pg/mL). Treatment with *T. purpurea* at 400 mg/kg significantly reduced TNF-α to 142.3±10.9 pg/mL (P<0.001), demonstrating potent anti-inflammatory activity. Similar patterns were observed for IL-1β and IL-6, with the highest dose showing reductions from 187.3±12.4 to 98.4±7.9 pg/mL and from 142.6±9.8 to 64.2±5.1 pg/mL, respectively. The dose-dependent inhibition of these key inflammatory cytokines suggests that *T. purpurea* exerts its anti-arthritic effects through modulation of pro-inflammatory signaling cascades, potentially involving NF-κB and other transcription factors.

Biochemical Parameters

Table 4: Effect of *T. purpurea* extract on biochemical parameters

Treatment Groups	CRP (mg/L)	RF (IU/mL)	AST (U/L)	ALT (U/L)
Normal Control	2.1±0.2	8.4±0.6	42.6±3.2	38.9±2.8
Arthritic Control	18.7±1.4	34.2±2.8	89.7±6.4	76.3±5.1
Diclofenac (10 mg/kg)	8.9±0.7***	18.6±1.9***	124.8±8.9**	118.4±7.6**
TP 100 mg/kg	15.2±1.2*	28.7±2.4*	67.4±4.8*	58.2±4.1*
TP 200 mg/kg	12.4±1.1**	23.5±2.1**	54.9±4.2**	49.7±3.8**
TP 400 mg/kg	9.8±0.8***	19.3±1.8***	47.8±3.6***	42.1±3.2***

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 compared to arthritic control.

The biochemical analysis demonstrates comprehensive anti-inflammatory effects of *T. purpurea* extract. C-reactive protein (CRP), an acute-phase inflammatory marker, was significantly elevated in arthritic animals (18.7±1.4 mg/L) compared to controls (2.1±0.2 mg/L). Treatment with *T. purpurea* at 400 mg/kg substantially reduced CRP levels to 9.8±0.8 mg/L (P<0.001), indicating systemic anti-inflammatory activity. Rheumatoid factor (RF), a specific marker for rheumatoid arthritis, was similarly reduced from 34.2±2.8 to 19.3±1.8 IU/mL. Importantly, *T. purpurea* treatment showed hepatoprotective effects, unlike diclofenac which caused significant elevation in liver enzymes. The extract at 400 mg/kg maintained AST and ALT levels close to normal values, suggesting safety for long-term use.

Hematological Parameters

Table 5: Effect of *T. purpurea* extract on hematological parameters

Treatment Groups	WBC (×10³/μL)	RBC (×10⁶/μL)	Hemoglobin (g/dL)	ESR (mm/hr)
Normal Control	7.2±0.5	7.8±0.4	14.6±0.8	8.4±0.7
Arthritic Control	16.8±1.2	5.9±0.4	10.2±0.6	42.6±3.1
Diclofenac (10 mg/kg)	10.4±0.8***	7.1±0.5***	13.8±0.7***	18.7±1.8***
TP 100 mg/kg	14.2±1.1*	6.4±0.5*	11.8±0.8*	36.4±2.7*
TP 200 mg/kg	12.6±0.9**	6.9±0.4**	12.7±0.7**	29.8±2.3**
TP 400 mg/kg	9.8±0.7***	7.4±0.4***	14.1±0.6***	21.3±1.9***

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 compared to arthritic control.

Hematological analysis reveals significant improvements with *T. purpurea* treatment, addressing systemic effects of arthritis. The arthritic control group showed marked leukocytosis (16.8±1.2×10³/μL) indicative of systemic inflammation, which was dose-dependently reduced by *T. purpurea* treatment, reaching near-normal levels (9.8±0.7×10³/μL) at the highest dose. Anemia, a common complication of chronic arthritis, was evident in the arthritic control group (hemoglobin: 10.2±0.6 g/dL) and significantly improved with treatment (14.1±0.6 g/dL at 400 mg/kg). The elevated ESR in arthritic animals (42.6±3.1 mm/hr) was substantially reduced to 21.3±1.9 mm/hr, indicating reduced systemic inflammation. These findings suggest that *T. purpurea* not only provides local anti-inflammatory effects but also addresses systemic complications associated with chronic arthritis.

Histopathological Examination

Table 6: Histopathological scoring of ankle joints

Treatment Groups	Synovial Inflammation	Cartilage Damage	Bone Erosion	Overall Score
Normal Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Arthritic Control	3.67±0.21	3.33±0.21	2.83±0.17	3.28±0.15
Diclofenac (10 mg/kg)	1.67±0.21***	1.83±0.17***	1.33±0.21***	1.61±0.14***
TP 100 mg/kg	3.00±0.26*	2.83±0.17*	2.50±0.22	2.78±0.17*
TP 200 mg/kg	2.33±0.21**	2.17±0.17**	1.83±0.17**	2.11±0.13**
TP 400 mg/kg	1.83±0.17***	1.67±0.21***	1.17±0.17***	1.56±0.15***

Scoring: 0=normal, 1=mild, 2=moderate, 3=severe, 4=very severe. Values are mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 vs arthritic control.

Histopathological examination provides crucial evidence of tissue-protective effects of *T. purpurea* extract. The arthritic control group exhibited severe synovial inflammation (3.67±0.21), extensive cartilage damage (3.33±0.21), and significant bone erosion (2.83±0.17), resulting in an overall pathological score of 3.28±0.15. Treatment with *T. purpurea* at 400 mg/kg dramatically reduced these parameters to 1.83±0.17, 1.67±0.21, and 1.17±0.17, respectively (all P<0.001), with an overall score of 1.56±0.15, comparable to diclofenac treatment (1.61±0.14). The dose-dependent improvement in histopathological parameters correlates well with clinical and biochemical findings, demonstrating that *T. purpurea* not only suppresses inflammation but also provides structural protection to joint tissues. This tissue-protective effect is crucial for long-term joint preservation and functional recovery.

6. Discussion

The present investigation provides comprehensive evidence for the potent anti-arthritic activity of *Tephrosia purpurea* extract in the Freund's complete adjuvant-induced arthritis model, which closely mimics human rheumatoid arthritis in terms of pathological features including synovial inflammation, cartilage destruction, and bone erosion (Geboes et al., 2007). The results demonstrate significant dose-dependent therapeutic effects across multiple parameters including clinical symptoms, inflammatory biomarkers, and histopathological changes.

Mechanism of Anti-Arthritic Activity

The observed anti-arthritic effects of *T. purpurea* can be attributed to multiple mechanisms of action. The significant reduction in pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 suggests modulation of key inflammatory pathways involved in rheumatoid arthritis pathogenesis (Comalada et al., 2005). These cytokines play crucial roles in synovial inflammation, cartilage degradation, and bone resorption through activation of matrix metalloproteinases and osteoclasts (Nair et al., 2007). The inhibition of these mediators by *T. purpurea* extract likely involves suppression of NF-κB signaling pathway, as demonstrated in previous studies with flavonoid compounds (Edwards et al., 2007). The phytochemical profile of *T. purpurea* extract, particularly the presence of flavonoids such as rutin and quercetin, provides insight into the molecular mechanisms underlying its therapeutic effects. Quercetin has been extensively studied for its anti-arthritic properties, demonstrating ability to inhibit TNF-α, IL-1β, and IL-6 production in activated macrophages and synoviocytes (Javadi et al., 2017). The compound also exhibits direct inhibitory effects on enzymes involved in inflammatory cascades, including cyclooxygenase and lipoxygenase (Jackson et al., 2006). Similarly, rutin has shown significant anti-inflammatory activity in adjuvant-induced arthritis models, reducing paw swelling and inflammatory mediator production (Guardia et al., 2001).

Comparative Efficacy and Safety Profile

The therapeutic efficacy of *T. purpurea* extract at 400 mg/kg was comparable to diclofenac sodium, a standard non-steroidal anti-inflammatory drug (NSAID) used in arthritis treatment. However, unlike diclofenac which caused significant hepatotoxicity as evidenced by elevated liver enzymes, *T. purpurea* treatment maintained normal hepatic function parameters. This favorable safety profile is consistent with previous toxicological studies reporting LD_{50} values exceeding 2000 mg/kg (Khatri et al., 2009). The hepatoprotective properties of *T. purpurea* have been documented in various experimental models, attributed to its antioxidant activity and ability to scavenge free radicals (Gora et al., 2014).

Systemic Anti-Inflammatory Effects

The hematological improvements observed with *T. purpurea* treatment indicate systemic anti-inflammatory activity beyond local joint effects. The normalization of white blood cell count, hemoglobin levels, and erythrocyte sedimentation rate suggests modulation of systemic inflammatory responses associated with rheumatoid arthritis (Saccol et al., 2019). Chronic inflammation in RA often leads to anemia of chronic disease and elevated acute-phase reactants, which were effectively addressed by the extract treatment.

Tissue-Protective Effects

The histopathological analysis revealed significant tissue-protective effects of *T. purpurea* extract, with preservation of cartilage architecture and reduced synovial hyperplasia. This chondroprotective activity may be mediated through inhibition of matrix metalloproteinases and inflammatory mediators that contribute to cartilage degradation (Yuan et al., 2020). The reduced bone erosion observed suggests potential effects on

osteoclast activity and bone remodeling processes, which are critically important for long-term joint preservation in rheumatoid arthritis.

Clinical Implications

The findings of this study have important clinical implications for the development of natural therapeutic agents for rheumatoid arthritis. The multi-target approach of *T. purpurea*, addressing inflammation, tissue protection, and systemic effects, offers advantages over conventional single-target therapies. The favorable safety profile makes it suitable for long-term use, which is essential for chronic conditions like RA. However, clinical trials in human subjects are necessary to establish therapeutic efficacy and optimal dosing regimens.

7. Conclusion

The present investigation demonstrates that hydro-alcoholic extract of *Tephrosia purpurea* leaves possesses significant anti-arthritic activity in the Freund's complete adjuvant-induced arthritis model. The extract showed dose-dependent therapeutic effects with significant reduction in paw swelling, arthritic scores, and inflammatory cytokines including TNF-α, IL-1β, and IL-6. Histopathological examination revealed substantial protection against synovial inflammation, cartilage damage, and bone erosion. Importantly, the extract exhibited a favorable safety profile with no adverse effects on hepatic function, unlike conventional NSAIDs. The therapeutic efficacy of *T. purpurea* at 400 mg/kg was comparable to diclofenac sodium, supporting its potential development as a natural therapeutic agent for rheumatoid arthritis. The presence of bioactive compounds such as flavonoids (rutin, quercetin) and their established anti-inflammatory properties provides mechanistic rationale for the observed effects. The multi-target approach of the extract, addressing both inflammatory processes and tissue protection, offers advantages over conventional single-target therapies. These findings provide scientific validation for the traditional use of *T. purpurea* in inflammatory joint disorders and support its potential development as a complementary or alternative therapy for rheumatoid arthritis management. However, clinical trials in human subjects are essential to establish therapeutic efficacy, optimal dosing, and long-term safety profile before clinical application.

8. References

1. Ahmad, V. U., Ali, Z., Hussaini, S. R., Iqbal, F., Zahid, M., Abbas, M., & Saba, N. (1999). Flavonoids of *Tephrosia purpurea*. *Fitoterapia*, 70(4), 443-445.
2. Akanksha, B., Avijit, M., Chakraborthy, G. S., & Seema, G. (2014). Phytopharmacological uses of *Tephrosia purpurea* - A review. *Pharmacophore*, 5(4), 658-665.
3. Chang, C. J., Tzeng, T. F., Liou, S. S., Chang, Y. S., & Liu, I. M. (2000). Flavonoids from *Tephrosia purpurea*. *Journal of Natural Products*, 63(12), 1679-1681.
4. Comalada, M., Camuesco, D., Sierra, S., Ballester, I., Xaus, J., Galvez, J., & Zarzuelo, A. (2005). In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. *European Journal of Immunology*, 35(2), 584-592.
5. Edwards, R. L., Lyon, T., Litwin, S. E., Rabovsky, A., Symons, J. D., & Jalili, T. (2007). Quercetin reduces blood pressure in hypertensive subjects. *Journal of Nutrition*, 137(11), 2405-2411.
6. Geboes, L., De Klerck, B., Van Balen, M., Kelchtermans, H., Mitera, T., Boon, L., De Wolf-Peeters, C., & Matthys, P. (2007). Freund's complete adjuvant induces arthritis in mice lacking a functional interferon-gamma receptor by triggering tumor necrosis factor alpha-driven osteoclastogenesis. *Arthritis & Rheumatism*, 56(8), 2595-2607.
7. Gokhale, A. B., & Saraf, M. N. (2000). *Tephrosia purpurea*: A review of contemporary literature and medicinal properties. *Ancient Science of Life*, 19(3-4), 168-173.
8. Gokhale, A. B., Damre, A. S., Kulkarni, K. R., & Saraf, M. N. (2002). Preliminary evaluation of anti-inflammatory and anti-arthritic activity of S. lappa, A. speciosa, A. aspera, T. dioica, T. purpurea and T. cordifolia in acute and chronic inflammatory models. *Journal of Ethnopharmacology*, 82(1), 67-70.

9. Gokhale, A. B., Damre, A. S., & Saraf, M. N. (2003). Investigations into the immunomodulatory activity of Argyreia speciosa. *Journal of Ethnopharmacology*, 84(1), 109-114.
10. Gora, R. H., Kerketta, P., Baxla, S. L., Toppo, R., Prasad, R., Patra, P. H., & Roy, B. K. (2014). Ameliorative Effect of *Tephrosia purpurea* in Arsenic-induced Nephrotoxicity in Rats. *Toxicology International*, 21(1), 78-83.
11. Guardia, T., Rotelli, A. E., Juarez, A. O., & Pelzer, L. E. (2001). Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Il Farmaco*, 56(9), 683-687.
12. Jackson, J. K., Higo, T., Hunter, W. L., & Burt, H. M. (2006). The antioxidants curcumin and quercetin inhibit inflammatory processes associated with arthritis. *Inflammation Research*, 55(4), 168-175.
13. Jain, A., Lodhi, S., & Singhai, A. K. (2009). Simultaneous estimation of quercetin and rutin in *Tephrosia purpurea* Pers by high performance thin layer chromatography. *Asian Journal of Traditional Medicine*, 4(3), 104-109.
14. Javadi, F., Ahmadzadeh, A., Eghtesadi, S., Aryaeian, N., Zabihiyeganeh, M., Rahimi Foroushani, A., & Jazayeri, S. (2017). The Effect of Quercetin on Inflammatory Factors and Clinical Symptoms in Women with Rheumatoid Arthritis: A Double-Blind, Randomized Controlled Trial. *Journal of the American College of Nutrition*, 36(1), 9-15.
15. Khatri, A., Garg, A., & Agrawal, S. S. (2009). Evaluation of hepatoprotective activity of aerial parts of *Tephrosia purpurea* L. and stem bark of *Tecomella undulata*. *Journal of Ethnopharmacology*, 122(1), 1-5.
16. Nair, M. P., Mahajan, S., Reynolds, J. L., Aalinkeel, R., Nair, H., Schwartz, S. A., & Kandaswami, C. (2007). The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-κβ system. *Clinical and Vaccine Immunology*, 13(3), 319-328.
17. Nile, S. H., & Park, S. W. (2014). Edible berries: bioactive components and their effect on human health. *Nutrition*, 30(2), 134-144.
18. Palbag, S., Dey, B. K., & Singh, N. K. (2014). Ethnopharmacology, phytochemistry and pharmacology of *Tephrosia purpurea*. *Chinese Journal of Natural Medicines*, 12(1), 1-7.
19. Rao, A. S., Yadav, S. S., Singh, P., Nandal, A., Singh, N., Ganaie, S. A., Yadav, N., Kumar, R., Bhandoria, M. S., & Bansal, P. (2020). A comprehensive review on ethnomedicine, phytochemistry, pharmacology, and toxicity of *Tephrosia purpurea* (L.) Pers. *Phytotherapy Research*, 34(8), 1902-1925.
20. Saccol, R. D. S. P., da Silveira, K. L., Adefegha, S. A., Manzoni, A. G., da Silveira, L. L., Coelho, A. P. V., & Leal, D. B. R. (2019). Effect of quercetin on E-NTPDase/E-ADA activities and cytokine secretion of complete Freund adjuvant–induced arthritic rats. *Cellular Biochemistry and Function*, 37(7), 474-485.
21. Singh, N., Gupta, S., Rathore, V., & Singh, S. K. (2014). Phytochemical and pharmacological review of *Cinnamomum verum* J. Presl - a versatile spice used in food and nutrition. *Food Chemistry*, 164, 180-190.
22. Winter, C. A., Risley, E. A., & Nuss, G. W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine*, 111(3), 544-547.
23. Yuan, K., Zhu, Q., Lu, Q., Jiang, H., Zhu, M., Li, X., Huang, G., Xu, A., Liu, L., & Zhang, C. (2020). Quercetin alleviates rheumatoid arthritis by inhibiting neutrophil inflammatory activities. *Journal of Nutritional Biochemistry*, 84, 108454.