Pharmacological spectrum of *Quercus infectoria* : (Comprehensive Review)

Ramakrishna S¹, Nisarga H L², Narayanamurthy G¹.

Ramakrishna.S, Assistant Professor, Department of Pharmacognosy, National College of Pharmacy, Shivamogga, Karnataka, India. Dr.NarayanaMurthy.G Principal& Professor, National College of Pharmacy, Shivamogga, Karnataka, India. Nisarga H L, UG Scholar, Department of Pharmacognosy National College of Pharmacy, Shivamogga, Karnataka, India. Corresponding author: Ramakrishna.S Assistant Professor, Department of Pharmacognosy, National College

of Pharmacy, Shivamogga, Karnataka, India.

Abstract:

Aleppo oak, or Quercus infectoria, is a common plant that has long been used in traditional medicine in many different cultures, especially in Ayurvedic and Unani medicine. A variety of pharmacological qualities are conferred by the physiologically active substances found in the plant, particularly in its galls, including hydrolysable tannins, gallic acid, ellagic acid, and flavonoids. Its Antibacterial, Anti-inflammatory, Antioxidant, Neuroprotective, Wound-healing, and Gastroprotective properties are all thoroughly examined in this overview of recent scientific research. Particular attention is paid to its Bioavailability, modes of action, synergistic effects in polyherbal formulations, and new developments in contemporary pharmacotherapy. r. The conversation highlights the gaps in current research and offers ideas for future lines of inquiry based on solid evidence. With the use of contemporary pharmacological research and traditional knowledge, this review seeks to provide readers a thorough grasp of Quercus infectoria's therapeutic potential and integrative medicine function.

Key words: Quercus infectoria, Neuroprotective, Wound-healing, Gastroprotective properties

Date of Submission: 12-05-2025	Date of acceptance: 26-05-2025

I. Introduction

Source: The oak species that is well-known for developing galls is *Quercus infectoria*, which belongs to the Fagaceae family.

This little tree may reach a height of one to two meters. Crooked, shrubby-looking stems have smooth, brilliant green leaves on short, 3–4 cm petioles. The bluntly murcronate, spherical, smooth leaves are uneven at the base and shining on the top.

Taxonomic Rank	Classification
Domain	Eukaryota
Kingdom	Plantae
Phylum	Tracheophytes
Subphylum	Angiospermae
Class	Rosids
Order	Fagales
Family	Fagaceae
Genus	Quercus
Species	Q. infectoria

Taxonomic Rank

Classification

Figure 1 Leaves & galls of Quercus infectoria



Pharmacological activities of Quercus infectoria :

1. Wound healing activity of Quercus infectoria gall extract

The goal of this study was to examine how topical administration of an ointment made from a hydroethanolic extract of Quercus infectoria gall affected the healing of open wounds in a model of diabetic BALB/c mice caused by streptozocin.

Following diabetes induction, the mice were split into three groups and had two circular (5 mm) lesions made on their dorsum. The mice received therapeutic amounts of 5% and 10% of an ointment made from Quercus infectoria, respectively, as well as soft yellow paraffin (control-sham group). Interleukin-6 (IL-6) and tumour necrosis factor (TNF)- α plasma levels, wound area, histological parameters, mRNA levels of vascular endothelial growth factor (VEGF), Bcl-2, and p53, and tissue antioxidant capacity were all examined in order to assess the therapeutic ointment's impact on the wound healing process.

Three equal groups of mice (n = 54) were created. Both ointment-treated groups showed a substantial reduction in wound area and TNF- α and IL-6 concentrations when compared to the control group (p<0.05). Additionally, there was a substantial (p<0.05) increase in angiogenesis, fibroblast dispersion per mm2 of wound tissue, collagen deposition, fast re-epithelialization, and VEGF, Bcl-2, and p53 mRNA expression. When compared to the control group, the ointment administration raised total antioxidant capacity and decreased malondialdehyde content (p<0.05).

According to our research, an ointment made from the hydroethanolic extract of Quercus infectoria gall sped up the healing of open wounds in a diabetic animal model by reducing the inflammatory phase, triggering apoptosis, increasing the expression of Bcl-2 and p53 mRNA, and promoting cellular proliferation and antioxidant qualities. 1

2. Anti bacterial activity :

Instead of killing the bacteria, the current study focusses on how extracts of Quercus infectoria gall, at subinhibitory concentrations, affect the related bacterial behaviours.

It was investigated how gall extracts affected the pathogenicity linked to quorum sensing (QS) in different drugresistant Pseudomonas aeruginosa collected from burn wounds. We looked at how various extracts affected the expression of genes encoding quorum sensing and exotoxin A, as well as the development of bacterial virulence and biofilm. In Pseudomonas aeruginosa and other medically relevant microorganisms, quorum sensing is an essential regulator of pathogenicity and biofilm formation. The acquired results showed that extracts of Q. infectoria galls either disrupt the pathogen's quorum sensing system by reducing virulence and bacterial coordination, or they combat infections by their inhibitory components, which actively eliminate cells [2].

3.Combined Antimicrobial Activity

Quercus infectoria (Q. infectoria) galls and Scrophularia striata (S. striata) aerial parts were chosen, and cariogenic microorganisms their antibacterial effectiveness against was examined. The antibacterial activity of water-soluble fractions derived from hydroalcoholic extracts of S. striata and Q. infectoria against Streptococcus mutans (S. mutans), Streptococcus sobrinus (S. sobrinus), and Candida albicans (C. albicans) was assessed both individually and in combination. After that, a herbal mouthwash was made using the extracts, and its stability and tannic acid concentration were assessed over a period of sixty days. S. striata extract improved the effective antibacterial activity of Q. infectoria gall extract in a synergistic manner. These extracts demonstrated antibacterial effectiveness, stability, and excellent organoleptic properties in mouthwash. Galls from S. striata and O. infectoria extracts

are capable of being combined to create dental products with potent anticariogenic qualities. When employing herbal products alone or in conjunction with other chemicals, our study emphasises the significance of thorough pharmacological examinations [3].

4. treatment of Gingivites and oral and pharyngeal muc

In-depth research on the characteristics of synthetic compounds is carried out concurrently with investigations into the efficacy and security of medications made from natural components. For many years, these medications have been successfully used in dentistry to treat oral cavity inflammation and in laryngology to treat pharyngeal inflammation. This article focusses on a collection of research on medications that use a composed ethanolic extract as an active ingredient. These extracts are derived from a variety of plants, including chamomile capitulum (Matricaria recutita L.), oak bark (Quercus spp.), sage leaves (Salvia officinalis L.), arnica herbs (Arnica spp.), calamus rhizomes (Acorus calamus L.), peppermint herbs (Mentha piperita L.), and thyme seeds. Preclinical research verified that a certain plant extract under test had antimicrobial properties (antibacterial, antifungal, and antiprotozoal). along with astringent, immunomodulatory, and anti-inflammatory qualities. It was verified in clinical settings that medications containing the extracted extract were used in periodontology, in the prevention and complex treatment of gingivitis, periodontitis, and diseases of the oral mucosa (but also the throat) caused by bacteria, viruses, fungi, and protozoa. These medications also significantly increased the effectiveness of therapy, reduced treatment duration, and enhanced the quality of life for patients. When utilised in dental surgery, the aforementioned preparations helped to speed up tissue regeneration and reduce pain and swelling following surgery or severe dentition. Moreover, medications containing the aforementioned extract may also be effectively utilised to decontaminate the oral cavity in cases of certain tooth or pharyngeal mucosal disorders. 4

5. Cytotoxicity of Quercus infectoria

Prior to further analysing the most effective extract for general phytochemical contents, the current work sought to assess the cytotoxic impact of QI extracts on a subset of human cancer cells. n-hexane (QIH), ethyl acetate (QIEA), and methanol (QIM) were the three primary extracts obtained from the sequential extraction of QI galls using n-hexane, ethyl acetate, and methanol. Qualitative analysis revealed the presence of tannin, alkaloids, glycosides, saponins, terpenoids, flavonoids, and phenolic chemicals in the most powerful extract. The MTT assay was then used to assess the extracts' cytotoxic activity against HeLa, MDA-MB-231, and Hep G2 cancer cells, which are breast, liver, and cervical cancer cells, respectively. The cytoselective ability of QI extracts was further assessed by evaluating their cytotoxic activity against the normal fibroblast (L929) cell line. DMSO-treated cells were used as the negative control, and cells treated with cisplatin were used as the positive control. Using Hoechst staining, the strongest extract was then selected to be examined further for DNA fragmentation, a sign of apoptosis. Through qualitative phytochemical examination, tannin, alkaloids, glycosides, saponins, terpenoids, flavonoids, and phenolic compounds were found. QIEA extract demonstrated cytoselective properties against L929 cells and the most cytotoxic effect against HeLa cells (IC50 value = $6.33 \pm$ 0.33 µg/mL). DNA fragmentation showed that QIEA caused the treated cells to undergo apoptosis. The abundance of phytochemical components in QIEA extract may be a factor in the strong cytotoxic effects on HeLa cells [5].

6. Anti colorectal cancer activity :

As an alternative treatment, Uyghur medicine's active component, Quercuse infectoria galls water extract (QIG), shows promise in treating Ulcerative Colitis (UC). Given the connection between colorectal cancer (CRC) and ulcerative colitis (UC), the current study intends to investigate the direct anti-CRC efficacy of

QIG extract. QIG extract was discovered to be able to cause caspase-dependent apoptosis and inhibit the viability of CRC cells. For the first time, we then demonstrated that QIG extract also caused autophagic cell death in CRC cells, which, when combined with apoptosis, added to the cytotoxic effect on CRC cells. Furthermore, it was discovered that intracellular ROS buildup linked to QIG-induced cytotoxicity may inhibit the AKT/mTOR signalling pathway.. then stop cell development and trigger autophagy. Additionally, the process of autophagic cell killing required the Erk signalling pathway. Furthermore, QIG extract reduced CRC cell migration and had an effect on the EMT process [6].

QIG as a substitute medication for the prevention and treatment of colorectal cancer.

7. irritable bowel syndrome :

Constipation, diarrhoea, and irritable bowel syndrome can be effectively treated with cinnamon bark and evergreen oak acorns, which are natural sources of beneficial components. Cinnamomum cassia bark (ACCE) and Quercus ilex aqueous extracts (GIAE) were evaluated for their different phytochemical compositions and probable opposing effects on gastrointestinal (GI)-physiological activities and disturbances. Using a test meal that contained charcoal and gum arabic in water using the phenol-red colorimetric technique, the GIphysiological effects of both extracts on gastric emptying (GE) and gastrointestinal transit time (GIT) were assessed. We investigated the opposite effects of ACCE/GIAE on GI diseases using colonic constipation and delayed stomach emptying caused by loperamide (LOP). It was shown by HPLC-PDA/ESI-MS that the primary phenolic chemicals found in ACCE are rosmarinic acid, quinic acid, trans-cinnamic acid, and protocatechuic acid; on the other hand, gallic acid, quinic acid, and protocatechuic acid are the main chemical components of GIAE. GIAE reduced GE at two dosages (150 and 300 mg kg-1) by 66.40% and 60.87%, respectively, in comparison to a control group (70.25%). Nevertheless, in rats, ACCE at the same dosages had opposing effects on the GE/GIT compared to GIAE and antagonistic synthetic pharmaceutical medications. In addition to its preventive action against rats' constipation caused by LOP, CCAE exhibited a potent antioxidant activity linked to the modulation of intracellular mediator abnormalities. Regarding the haematological profile and biochemical markers, no harmful activities were found.

8. anti-herpetic effect :

We investigated the anti-herpes action in vivo of castalagin, a ellagitannin compound that was isolated from Quercus robur, or pedunculate oak. Prior studies have demonstrated that castalagin exhibits an in vitro potent inhibitory effect against HSV-1/2 comparable to that of acyclovir (ACV). Additionally, it works well against mutants that are resistant to ACV and has a synergistic effect with ACV. In neonatal mice, we investigate the effect of castalagin on HSV-1 infection. Mice with acute poisoning had an LD50 value of 295 mg/kg. Long-term toxicity was also created. When given as 0.02 ml s.c. dosages of 7.5 or 10 mg/kg during a 7-day term, castalagin demonstrated a notable efficacy against HSV-1 (LD90/0.02 ml) (PI 57-58%). ACV course showed significant efficacy at 20 mg/kg. The LD50/ED50 (295/7.5) selectivity ratio may be regarded as > 33 [8].

9. topical antiaging formulation :

It has been found that Quercus infectoria Olivier (galls) has astringent, antidiabetic, antipyretic, antitremor, local anaesthetic, and anti-parkinsonian pharmacological aspects. In Asian countries, traditional eastern medicine has utilised Quercus infectoria galls for millennia to treat inflammatory diseases. Creating a stable water-in-oil (w/o) emulsion of Quercus infectoria Olivier gall extract and evaluating its impact on skin mechanical characteristics and antiaging effects were the study's goals. The findings demonstrated that Quercus infectoria Olivier possesses antioxidant and sun protection qualities (SPF), lowers sebum, improves elasticity, and produces a stable emulsion with 4% Quercus infectoria gall extract that may be used topically as an antiaging product.

[9] (dental pathogens).

10. anti-bacterial agents :

Nowadays, many microorganisms are resistant to antibiotics. The significance of ayurvedic medications is heightened by this. The activity of several extracts of Quercus infectoria galls (petroleum ether, chloroform, methanol, and water) against dental pathogens, including Streptococcus mutans, Streptococcus salivarius, Staphylococcus aureus, Lactobacillus acidophilus (designated), and Streptococcus sanguis (isolated), is reported here. In conclusion, the plant may produce metabolites that are herbal. The development of new chemical families of antibiotics may be the outcome of the crude extracts exhibiting anti-dental caries action. These antibiotic chemical families have the potential to be selective agents for maintaining human health and to offer biochemical tools for studying infectious illnesses [10].

11. Anti inflamatory activity :

Inflammatory processes have been demonstrated to be downregulated by Quercus infectoria (Qi) extract; however, the molecular mechanisms underlying this anti-inflammatory action are yet unknown. We hypothesised that Qi extract reduces inflammation via inhibiting the Set7/NF- κ B pathway. To replicate the diabetic milieu, bone marrow-derived macrophages (BMM) were incubated with high glucose plus palmitate medium (HG/Pa). In contrast to control circumstances, HG/Pa increased the expression of many inflammatory cytokines, Set7, and NF- κ B activity and expression. These alterations were linked to elevated intracellular reactive oxygen species (ROS) levels. Additionally, comparable changes were shown in BMM from mice fed a high-fat diet (HFD) as opposed to lean animals. indicating that BM progenitors' alterations brought on by HFD last throughout differentiation and culture. Crucially, in both HG/Pa-treated and HFD-treated BMM, Qi extract dose-dependently decreased the expression of Set7, p65, and inflammatory cytokines in comparison to vehicle controls. Lastly, the inflammatory cytokines IL-1 β and TNF- α were expressed at lower levels by macrophages/monocytes isolated from diabetic mice's wounds treated with Qi solution than by wounds treated with a vehicle, indicating a translation to the in vivo diabetic milieu. Together, the findings of this study indicate that Qi inhibits the Set7/NF-kB pathway's activation brought on by diabetes. 11

12. HIV-1 reverse transcriptase inhibitory activity

Since it is in charge of converting viral RNA genomes into DNA genomes, the HIV-1 reverse transcriptase (HIV-1 RT) has emerged as a key target for HIV infection therapy. Peptides that have been hydrolysed from plants are thought to be a novel source of possible medications. 111 Asian medicinal plants' peptides were tested against the HIV-1 RT in attempt to create novel, potent inhibitors. Using HPLC and LC-MS, the crude hydrolysed peptides from Quercus infectoria fruit peel were chosen for purification and peptide sequence analysis. The capacity of two synthesised peptides of interest to inhibit the HIV-1 RT was assessed using an IC50 assay. The peptides LIAVSTNIIFIVV and AIHIILI were found to have respective IC50 values of 236.4 \pm 7.07 nm and 274 \pm 5.10 nm. This suggested that these peptides may be produced further as possible inhibitors of HIV-1 RT.

13. anti microbial agent

The antibacterial activity of Q. infectoria extract (QIE) was evaluated against a variety of microbiological species and used to the cleaning of eggshell. With several test techniques, QIE's antibacterial activity was demonstrated against Salmonella Typhimurium, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Candida albicans. The total colony count of yeasts, moulds, and Enterobacteriaceae was significantly decreased by immersing eggshells in 1% QIE solution to disinfect them of microbial contamination. Following a 60-minute immersion in QIE, S. aureus and E. coli were totally suppressed. Elevated levels of flavonoid and phenolic chemicals were found by QIE biochemical examination. Strong morphological changes were seen in the micrographs of S. aureus cells treated with QIE; after six hours of treatment, the cells were completely lysed and burst. When it comes to decontaminating eggshells, QIE is a natural and efficient disinfectant.

14. Anti fungal activity

The objective of this study was to examine the antifungal properties of phenolic alcohol extract for the dry plants Bitter Melon (Citrullus colocynthis (L.) Schrad) and Oak (Quercus infectoria Oliv). To combat two fungus, Penicillium expansum and Aspergillus flavus, three concentrations of phenolic alcohol extract of bitter melons and oak (100, 200, and 300 mg/mL) have been developed. According to the data, phenolic extracts exhibited antifungal efficacy at all three doses, and as concentrations rose, so did the percentage inhibition of diameter growth (PIDG). When tested against P. expansum and A. flavus, the extract from C. colocynthis produced the greatest average of PIDG (38.29%), followed by that of Q. infectoria (34.13%). The fungus A. flavus was more strongly inhibited. as opposed to P. expansum, which had an average PIDG of 23.37%, with an average of 49.05 percent. At a concentration of 300 mg/mL, the results indicated that the extract from C. colocynthis produced the greatest PIDG (70.7 \pm 3.90) on P. expansum, followed by Q. infectoria with PIDG (31.1 \pm 3.335). In contrast, the antifungal activity of C. colocynthis extract had the greatest PIDG (72.09 \pm 4.10) while Q. infectoria had the second-highest PIDG (62.49 \pm 3.63) at a concentration of 300 mg/mL, according to the results for phenolic extracts of both species on A. flavus. The fruit of Q. infectoria galls and C. colocyn exhibited inhibitory action against two toxin-producing fungus, P. expansium and A. Flavus, according to our findings.14

15. Atherosclerotic

Numerous problems in human cultures are brought on by hyperlipidaemia. This study examined the impact of methanol extracts of Rosa damascena (RD) Mill flower and Quercus infectoria (QI) galls on the development of atherosclerotic plaques and lipid profiles in hyperlipidemic rabbits. For forty-five days, thirtysix New Zealand white rabbits were randomly assigned to six groups: control (I), hyperlipidemic (II), hyperlipidemic+RD (IV), +Atorvastolin (V), and hyperlipidemic+Orlistat (VI). They were fed a high-fat diet consisting of 0.5% cholesterol and 16% hydrogenated vegetable oil. Plaque development and the lipid profile were evaluated at the conclusion of the research period. In comparison to the control group, the hyperlipidemic group's levels of total cholesterol (TC), low density lipoprotein (LDL), and triglycerides (TG) were considerably higher (p < 0.001). Consumption of Quercus infectoria methanol extract dramatically reduced TC, TG, and LDL plasma levels (p < 0.001). Additionally, it reduced the development of plaque in the thoracic aorta and semi-lunar valve. The methanol extract from Rosa damascena mill flowers reduced TC, TG, LDL, and plaque formation levels somewhat but not significantly. Animal weights and HDL levels did not significantly differ across groups. Our results showed that QI but not RD methanol extract had anti-atherogenic and hypolipidemic properties based on the dosages utilised in this investigation.

16. Anti amoebic activity :

The study examined the anti-amoebic properties of crude methanol extracts of Quercus infectoria nut gall, Piper sarmentosum root, and Piper longum fruit against Entamoeba histolytica infections in mice's caecum. By injecting Entamoeba histolytica trophozoites straight into the caecum, mice were made to get caecal amoebiasis. After the infection, the mice were given the extract orally for five days in a row, starting 24 hours later, or a normal medication (metronidazole) or vehicle p.o. They were then assessed on the sixth day. The fruit extract of Piper longum, the root extract of Piper sarmentosum, and the nut gall extract of Quercus infectoria showed curative rates of 100, 40, and 26% at a daily dosage of 1000 mg/kg. While extract from the root of Piper sarmentosum at a dose of less than 1000 mg/kg per day could not cure any mice from amoebiasis, extract from the fruit of Piper longum remained beneficial in 93 and 46% of the cases at concentrations of 500 and 250 mg/kg/day, respectively. At 500 and 250 mg/kg per day, respectively, extract of Quercus infectoria nut gall healed 26 and 13% of mice. Curative rates for metronidazole were 100% and 60% at concentrations of 125 and 62.5 mg/kg daily, respectively. Compared to the control group, mice who got the extract plus metronidazole had less severe caecal wall ulceration.

II. Conclusion

A combination of historical usage and scientific confirmation supports Quercus infectoria's broad medicinal potential. It is a strong contender for natural medicine development because of its bioactive components, which have shown encouraging therapeutic benefits. Its antibacterial, anti-inflammatory, and antioxidant qualities are well established, but further study is necessary to improve its pharmacokinetics, determine the ideal therapeutic dose, and evaluate its long-term safety through thorough clinical studies. Incorporating Quercus infectoria into pharmaceutical and cosmetic applications may improve its availability and effectiveness in contemporary medical environments. By providing innovative approaches to illness treatment, Quercus infectoria may be able to close the gap between modern pharmacology and herbal medicine. Future research should concentrate on clinical efficacy evaluations, bioavailability improvement techniques, and molecular pathways to fully use its therapeutic qualities

Bibliography:

- [1]. Dard Mark F,et al. Quercus infectoria gall extract aids wound healing in a streptozoan-induced diabetic mouse model. 2021;518-625.
- [2]. Ahmed A. Quercus infectoria gall extracts subdue quorum sensing-controlled virulence factor production and biofilm formation in Pseudomonas aeruginosa recovered from burn wounds. 2019;1-2.
- [3]. Falakdin P. Combined antimicrobial activity of extracts from Quercus infectoria galls and Oberophe laria striata aerial parts for an anticariogenic herbal mouthwash. Pharmacopunch. 2023;44-52.
- [4]. Schonknecht K, et al. Effectiveness of composed Huubal extract in the treatment of gingivitis and oral and pharyngeal mucosa. Wiad Lek. 2021;1737-1749.
- [5]. Wan Yusof WNB, et al. Phytochemical and cytotoxicity analysis of Quercus infectoria ethyl acetate extracts on human cancer cells. Trop Life Sci Res. 2020;69-84.
- [6]. Zhang H, et al. Traditional Uighur medicine Quercus infectoria galls water extract triggers cell death via apoptosis and autophagy in colorectal cancer cells. BMC Complement Med Ther. 2020;PMID: 33272262, PMC7712637.
- [7]. Sebai H, et al. Modulating and opposite actions of Cassia L. bark and Quercus ilex L. aqueous extract on the gastrointestinal tract in rats. RSC Adv. 2019;38:21695-21706.
- [8]. Stoyanova A, et al. Effect of castalagin against HSV-1 infection in newborn mice. Nat Prod Res. 2023;4156-4161.
- [9]. Choudry A, et al. Formulation and characterization of Quercus (Olivier) emulsions and in vitro, in vivo evaluation as a cosmeceutical formulation. Cosmet Dermatol. 2023;3480-3490.

- [10]. Vermani A, et al. Screening of Quercus infectoria gall extracts as antibacterial agents against dental pathogens. Indian J Dent Res. 2009;337-339.
- [11]. Chokpaisarn J, et al. Quercus infectoria inhibits the ASET 7/NF-κB inflammatory pathway in macrophages exposed to a diabetic environment. Cytokine. 2017;29-36.
- [12]. Seetaha S, et al. Novel peptides with HIV-1 reverse transcriptase inhibitory activity derived from the fruits of Quercus infectoria. Chem Biol Drug. 2021;157-166.
- [13]. Tayel A, et al. Application of Quercus infectoria extract as a natural antimicrobial agent for chick egg decontamination. Rev Argent Microbiol. 2018;391-397.
- [14]. Kadam SW, et al. Antifungal activity of phenolic compounds separated from Quercus infectoria and Citrullus colocynthis against toxic fungi. Arch Razi Inst. 2023;297-303.
- [15]. Gholamhoseinian A, et al. Effect of Quercus infectoria and Rosa damascena on lipid profile and atherosclerotic plaque formation in a rabbit model of hyperlipidemia. Pak J Biol Sci. 2012;27-33.
- [16]. Sawangjaroen N, et al. Effects of Piper longum fruit, Piper sarmentosum root, and Quercus infectoria nut gall on caecal amoebiasis in mice. Ethnopharmacol. 2004;357-360.