*Strobilanthes barbatus*ameliorates adjuvant-induced arthritis in rats through inhibiting TNF-α signaling pathways

Farzana Hilal[1], Aysha O.S[2]
Research Scholar, Department of Microbiology, Mohammad Sathak College, Sholinganallur, Chennai, Tamilnadu, India
Head of the Department of Microbiology, Mohammad Sathak College, Sholinganallur, Chennai, Tamilnadu, India

Abstract -*Strobilanthes **barbatus*** **belongs to the family Acanthaceae, is an indigenous plant. The plants of this family possess various pharmacological activities such as anti-inflammatory, antioxidant, anti-osteoarthritis, neurological disorder, anti-diabetic and anti-microbial. The plant has not been investigated for its anti-inflammatory and antiarthritic activity. The present study was undertaken to explore its possible anti-inflammatory and antiarthritic activity. Anti-inflammatory activity of alcoholic and aqueous extracts of the leaves was assessed by in vivo methods. This study was evaluated to show that the *S.barbatus* leaves extracts possess anti-inflammatory activity and have pronounced effects on adjuvant arthritis. Adjuvant (CFA)-induced arthritis (AIA) was developed and used to test the efficacy of *S.barbatus* on arthritis rats. Rats were given an oral dose of (75mg/kg, 150mg/kg and 300mg/kg,) once daily from days 14 to 28 after the administration of CFA. The pro-inflammatory cytokine levels TNF alpha level in serum were detected by ELISA and Biochemical markers were also estimated for the *S.barbatus*. X- Ray and HE staining was used to determine representative histological changes in joint tissues. *S.barbatus* suppressed the release of tumor necrosis factor-alpha. In addition, *S.barbatus* could also dramatically ameliorate the pathological changes observed in rat joints. Based on the results, we also observed the changes in biochemical expression by reduction of inflammation. These results proved that treatment with *S.barbatus* is significantly effective for arthritis and inhibiting the TNF alpha signaling pathway may be a potential therapeutic target for treatment of arthritis.**

Index Terms **- Arthritis, inflammation, TNF alpha pathway, Inflammation, Plant extract, Adjuvant-induced arthritis, Rheumatoid arthritis.**

I.INTRODUCTION

Rheumatoid arthritis (RA) is a systemic and chronic inflammatory autoimmune disease characterized by chronic inflammation, synovial hyperplasia with concomitant joint destruction, deformity, and loss of function. (Liet al., 2017) Although the exact pathogenesis and etiology of the disease remain unclear, the main pathological changes have been defined, such as abnormal immunity, chronic synovitis, inflammatory cell infiltration, pannus formation, destruction of cartilage and bone erosion (Liet al., 2005). The development of RA involves a complex interplay of several types of cells, including B and T lymphocytes, macrophages, fibroblastlike synoviocytes, endothelial cells and dendritic cells. Notably, B and T cells play critical roles in the pathogenesis (Huang et al.,1998). Currently, the clinical need for effective treatment of RA remains unmet and more novel drugs are highly demanded. Strobilanthes spp is one of the endemic and potential medicinal plants. It is widely used in Ayurveda as a source of the drug 'Sahacharya' (Greenwald, 1991). Strobilanthes species are known to exhibit various biological activities such as antiviral, antifungal, acetylcholine esterase inhibitory, anti-obesity, anti-inflammatory, anticancer, antioxidant, antidiabetic, antinociceptive and hypoglycemic activity (Greenwald et al., 1991).

Rat adjuvant arthritis (AA) is a chronic, polyarticular, erosive type of arthritis induced by an injection of killed mycobacteria AA in rat is an experimental model that shares some features with human rheumatoid arthritis (RA) (Schmidt et al., 1999) . One

of the most important features of AA is the chronic synovitis, including inflammatory cell infiltration, pannus formation, cartilage destruction and bone erosion. AA is widely used for studying the pathogenesis of RA and for searching new drugs for treatment of rheumatoid disease (Zheng et al., 2005).

TNF- α can activate NF-κB by degrading IκB, its inhibitory protein (Zhang et al., 1990). However, the effect of *Strobilanthes barbatus* on (Rheumatoid arthritis) is not known. Hence the aim of the present study was to investigate the effect of *Strobilanthes barbatus* on Rat adjuvant arthritis (AA) inflammation and evaluate the significant change in TNF-α pathways (Noguchi et al.,2005).

II.MATERIAL AND METHODS

2.1 Collection, identification, and extraction of plant material

The plant *Strobilanthes barbatus* collected in June 2017 from Amruth Vana – FRLHT's Ethanomedicinal Garden, Attur, Yelahanka, Bengaluru. The plant was identified and authenticated by a Ethanobotanist, (Dr. Ganesh Babu, Head, Department of Herbal Gardens Sciences, Bangalore, Karnataka. The fresh green aerial parts of the plant were collected and were dried at room temperature under shade for five days. This dried powdered was subjected to various extraction using different solvent in Soxhlet apparatus. Then the extract was evaporated to dryness using Bauchi Rotavapor (Switzerland) and ultimately dried in an oven.

2.2 Drugs and reagents

Dexamethasone was obtained from Sigma-Aldrich India. *Strobilanthes barbatus* or Dexamethasonewas ground and suspended in normal saline containing 0.5% sodium carboxymethyl cellulose (CMC) for administration.

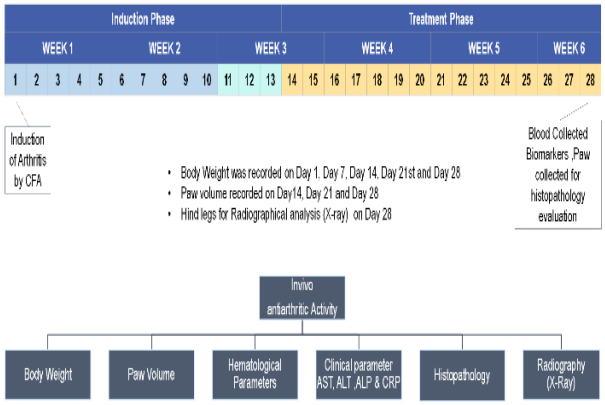
2.3 Animals

SD rats weighing 160±20 g was obtained from animal house of Sathya Bama Institute of Science and Technology, Chennai and were acclimatized to the housing conditions for 7 days with access to laboratory chow diet and water ad libitum. Animal requirement was approved by the Institute of Animal Ethics Committee (SU/CLATR /IAEC/XI/104/2018, and all the experiments were conducted as per the norms of the committee for the purpose of supervision of experiments on animals.

2.4 Induction of adjuvant arthritis

Adjuvant arthritis was induced by an injection of Complete Freund's adjuvant, (Jinet al., 2002) the rats were immunized with 0.1 ml of CFA (CFA: 10 mg/ml; Sigma-Aldrich, USA into the base of tail (day 0). Fourteen days after inoculation, the animals were selected and distributed into groups (n =6) according to the severity of arthritis, so that each group had similar disease severity at the beginning of the treatment. One was given 0.5% CMC solution as vehicle-treated group, the others were given *Strobilanthes barbatus* (75, 150 and 300 $mg/kg^{-1}/day^{-1}$), or Dexamethasone ($10mg/kg^{-1}/day^{-1}$) intragastrically from day 14 to day 28, respectively.

Figure 1: Experimental Procedure



2.5 Measurement of paw volume&Body weight

The severity of AA was quantified by measuring the volume of hind paws using a water plethysmometer. Paw volume (ml) was measured on days 0,7,14 and 28 after arthritis induction (Kalyan kumar et al.,2015). Data were expressed as the volume of increase with respect to day 0 volume.Body Weight was recorded on Day 1, Day 7, Day 14, Day 21and Day 28

2.6 Hematological &Biochemical Parameter

On the last day of the study (day 28), blood was drawn using the retro-orbital puncture method and was collected into vials containing EDTA (Xu et al., 1991). Red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) concentration, and

erythrocyte sedimentation rate (ESR) were evaluated at VRR Diagnostics. Collected blood of rats in all experimental groups without anticoagulant and was centrifuged at 3,000 rpm, 4°C for 10 min at day 28 after treatments. The serum was separated and divided into aliquots at 4°C. The serum levels of the arthritis factor (CRP), C-Reactive Protein, alkaline phosphatase (ALP), amino transaminase (AST), and alanine amino transaminase (ALT) were investigated by commercially available colorimetric assay kits in VRR Diagnostic.

2.7 Histopathological &Radiological analysis of ankle joints

On day 28, rats were anaesthetized, and radiographs of the adjuvant injected hind paws were taken using X-ray (AGFA CR 30-Xunit, Germany). Radiographic analysis of hind paws was performedat 55kV peak, 50mA and the exposure time was 5s.

On day 28, ankle joints were separated from the hind paw and immersed in 10% buffered formalin for 24h followed by decalcification in 5% formic acid, processed for paraffin embed-ding sectioned at 5µthickness. The sections were stained withhematoxylin-eosin and evaluated under light microscope with10×magnifications for the presence of inflammatory cells, hyperplasia of synovium, pannus formation and destruction of joint space

2.8Assay of myeloperoxidase in rat blood serum

The blood was collected in a centrifuge tube and kept for 30 min undisturbed at room temperature. Then centrifuged at 1000-2000 g for 10 min to remove the clot and serum was collected. Myeloperoxidase activity in serum was determined by (Bradley et al. 1982).

2.9Whole blood assay TNF-α (LPS Induced)

SD rats weighing 160±20 g was fasted overnight and intraperitoneally injected with lipopolysaccharide (LPS, Escherichia coli serotype 055: B5, Sigma-Aldrich) dissolved in saline (300µg/kg). *Strobilanthes barbatus* (75, 150 and 300 mg/kg) was administered orally 60 min prior to LPS injection. Serum was collected 1 h after LPS injection. The amounts of TNF-α in serum were measured by Enzyme Linked Immunosorbent Assay (ELISA)*He et al., 1991).

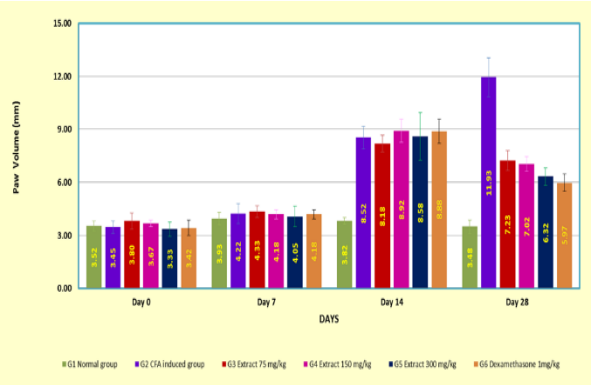
Statistical analysis

Data was expressed as Mean±SEM and statistical analysis wascarried out by using GraphPad 5.0 software (GraphPad, San Diego),USA) by applying two-way ANOVA with Bonferroni test or onewayANOVA with Dunnett's test<0.05 was significant.

III.RESULTS AND DISCUSSION

Effects of *Strobilanthes barbatus* on paw swelling in adjuvant arthritis rats was induced reproducibly in all animals injected with the adjuvant, with onset of erythema and swelling (arthritis onset) occurring on day 14 and persisted to the end of the experiment. Treatment with *Strobilanthes barbatus* (75, 150, 300 mg kg^{-1} day^{-1}, days 14–28) and Dexamethasone (1 mg $kg^{-1}day^{-1}$) diminished the paw swelling from day 20 to 28 ($P < 0.05–0.001$)

Figure 1A: Paw Volume



Body weight results are shown in Fig. 1B. Compared with the normal group, the body weight of FCA model group rats significantly ($P < 0.05, 0.01$) decreased from 10 to 14 days. Compared with the FCA model group, *Strobilanthes barbatus* (300 mg/kg) and *Strobilanthes barbatus* (150 g/kg) significantly ($P < 0.05, 0.01$) increased body weight in AIA rats during 16-28 days and 20-28 days, respectively. *Strobilanthes barbatus* (300 mg/kg) significantly ($P < 0.05, 0.01$) increased body weight at 24, 28 days. The body weight of Dex (1mg/kg) had significant ($P < 0.01$) reduction from 14 to 28 days. reduced paw swelling volume in rats from 4 to 28 days.

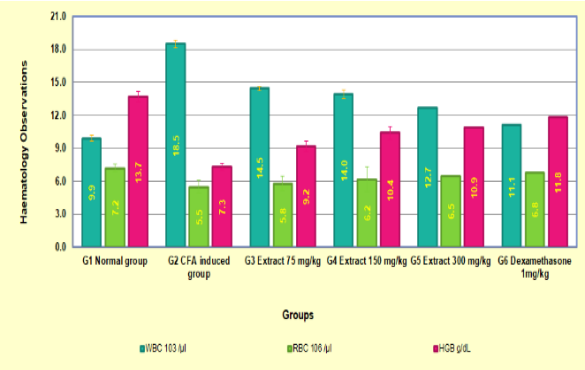
Figure 1B: Body Weight

Effect of Hematological parameter

The current results demonstrated that the arthritic rats showed a rise in WBC's count along with reduced RBC's count, Hb concentration, platelet count, and HCT value in comparison with vehicle control rats. All these measured parameters indicate the anemic condition commonly noted in arthritis. Both implemented treatments ameliorated the anemic condition as marked by reversing back WBCs count and increasing RBCS count, HB concentration, and HCT value compared to AIA group. The CFA induced group showed low levels of RBC, HGB and elevated levels of WBC. The treatment with extract at different doses has shown marked effect on the above parameters. However, the group with dose of 300mg/kg has better effect compared to other groups. The effect observed was almost comparable to the standard group.

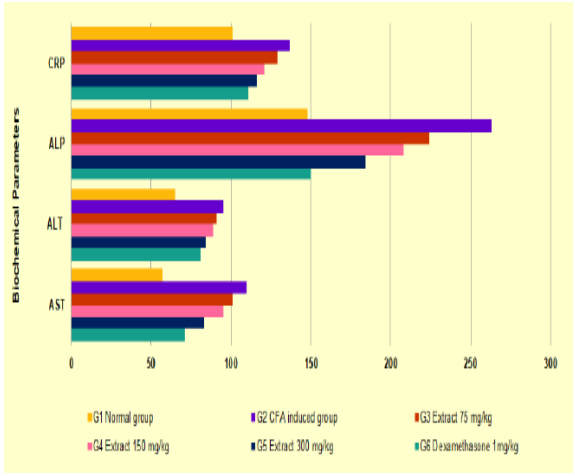
On the other hand, the rats treated with Dexamethasone resulted in slight increase in platelet count while treatment with *Strobilanthes barbatus*showed no effect on platelet count compared to AIA group (Figure 2).

Figure 2: Hematology Parameter



Arthritic biomarkers like C-reactive protein (CRP), rheumatoid factor (RF), and myeloperoxidase activity were clinically evaluated in rat serum. It was observed that CRP and RF values were increased in RA condition of AIA rats and this change was significant ($p < 0.05$) when compared to normal. At the same time in groups IV and V *Strobilanthes barbatus*from 14th day showed highly significant decline in the level of these parameters as compared to untreated rats (II group) (Figure:3). The biochemical parameters of the serum from CFA induced group showed significantly high values of AST, ALT, ALP and CRP. The groups G5 and G6 showed better inhibition compared to other groups.

Figure 3: Biochemical Parameter



Myeloperoxidase enzyme activity was assessed in serum to measure infiltration rate of neutrophils. In AIA rats enhanced MPO activity was observed as indicated in Table 1 and however upon *Strobilanthes barbatus* administration strongly inhibited enzyme activity in a significant manner ($p < 0.05$).

Table 1: MPO Activity

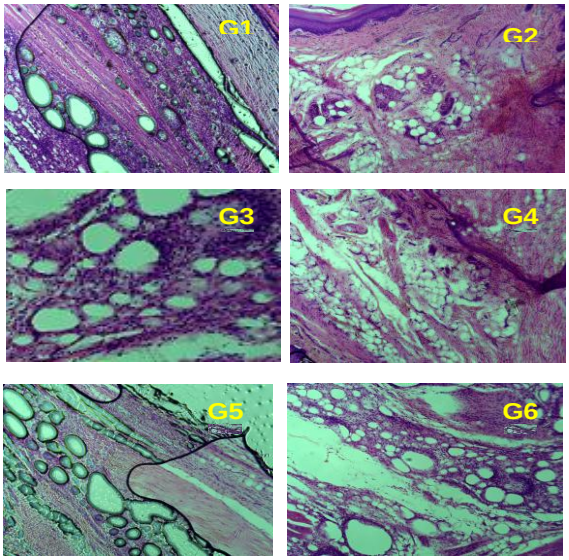
G. No	MPO (µg/ml)	Mean	SD
G1	Normal control	0	0
G2	CFA induced	965	42.78
G3	Extract 75 mg/kg	861	23.29
G4	Extract 150 mg/kg	749	54.82
G5	Extract 300 mg/kg	554.8	26.1
G6	Dexamethasone 1mg/kg	410.9	31.92

Histopathology

The histopathology studies of hind paw joints in the CFA induced group showed abnormalities like extensive cellular infiltration and destruction of bone

marrow, as compared to the normal group. The groups G5 and G6 did not show the cellular infiltration and destruction of bone marrow.

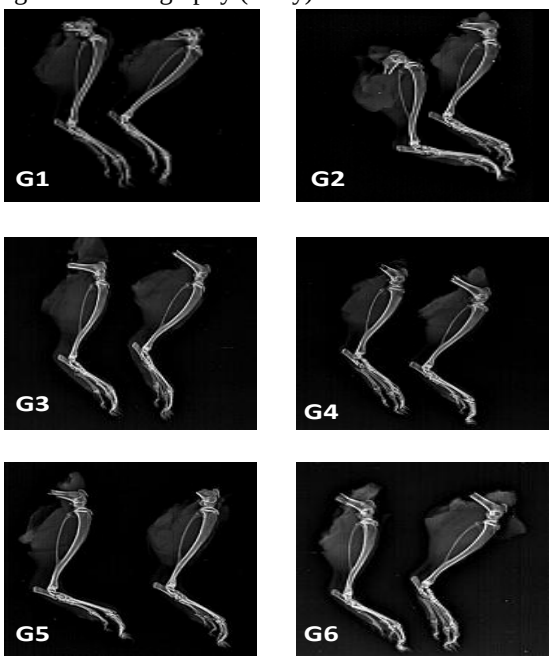
Figure 4: Photomicrographs for joint sections stained with H& E



Radiological study

The x-ray of the paw joints showed a soft swelling of tissues and bone destruction in the CFA induced group. Bone destruction was not observed in the standard group. The groups G5 and G6 showed better effect, by prevention of bone destruction, compared to other treatments.

Figure 4: Radiography (x-ray)



Effects of *Strobilanthes barbatus* on Whole blood assay - LPS-induced TNF-α production in rats

Amount of plasma TNF-α were measured1hour after LPS injection since TNF-α level were maximal at this time. The average baseline TNFα levels were 2523.17 pg/ml in rats. As displayed in (Table.2) *Strobilanthes barbatus* (#75, #150,#300mg/kg, per oral resulted in dose-dependent inhibition of LPS-induced TNF-α productionstatistically estimated using Sigmoidal dose-response (variable slope) by best fit value to calculate inhibition using graph prism.

Table 2: Tumor Necrosis Factor alpha (TNF-α) Activity

G.No	TNF -Alpha(pg/ml)	Mean	SD
G1	Normal control	0.00	0.00
G2	LPS induced	2523.17	187.23
G3	Extract 75 mg/kg	2143.67	141.34
G4	Extract 150 mg/kg	1803.33	113.15
G5	Extract 300 mg/kg	1323.00	76.53
G6	Dexamethasone 1mg/kg	973.17	73.87

Tumor Necrosis Factor alpha (TNF-α) is a ubiquitous, multifunctional cytokine produced primarily by activated monocytes, macrophages and T-cells, and plays a key role in fighting infection, eradicating tumors, and mediating the acute and chronic inflammatory effects of the immune system. Excessive production of TNF-α, however, has been directly implicated in a wide variety of diseases that differ considerably in their etiology and clinical manifestations. Over the past decade, the important contributory role of TNF-α plays in the diverse diseases like RA.

IV.CONCLUSION

On evaluating the antiarthritic activity of *Strobilanthes barbatus*, the effect of the extract at 300 mg/kg was found to be higher especially in the developing phase of arthritis. In the light of the above results, it might me concluded that the *Strobilanthes barbatus* exhibited a potent antiarthritic effect by reducing the pathological lesions via down regulating the levels of proinflammatory cytokines TNF alpha thereby reducing the levels of acute phase proteins and via its enhanced immunomodulatory property on humoral and cellular immune responses. From the

present study, we found that *Strobilanthes barbatus* has therapeutic effect on AIA, and the effect might relate to its suppressive effect on the production of pro-inflammatory cytokines and on humoral and cellular immune responses However, further studies are necessary to identify the active phytoconstituent responsible for the antiarthritic activity. This can be studied in future to develop it as an alternate treatment for adjuvant induced arthritis.

REFERENCES

[1] T. Pan, T.F. Cheng, Y.R. Jia, P. Li, F. Li, Anti-rheumatoid arthritis effects of traditional Chinese herb couple in adjuvant-induced arthritis in rats, Journal of ethnopharmacology 205 (2017) 1-7.

[2] Bradley PP, Pribat DA, Christensen RD, Rothestein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Clin Invest Dermatol 1982;78:206-9.

[3] Greenwald, R.A., Animal models for evaluation of arthritis drugs.Methods Find Experimental and Clinical Pharmacoloy 1991; 13: 75–83.

[4] Schmidt-Weber, C.B., Pohlers, D., Siegling, A., Schadlich, H., Buchner, E., Volk, H.D., P.Kinne, E., Emmrich, F.,W.Kinne, R. Cytokine gene activation in synovial membrane, regional lymph nodes, and spleen during the course of rat adjuvant arthritis. Cellular Immunology 1999;195:53–65.

[5] Zheng,Y.Q.,Wei,W. Total glucosides of paeony suppresses adjuvant arthritis in rats and intervenes cytokine-signaling between different types of synoviocytes.International Immunopharmacology 2005;5:1560–1573.

[6] Turull, A., Queralt, J. Selective cyclooxygenase-2 COX-2 inhibitors reduce anti-Mycobacterium antibodies in adjuvant arthritic rats. Immunopharmacology 2000; 46:71– 77.

[7] Jin, H.Z., Hwang, B.Y., Kim, H.S., Lee, J.H., Kim, Y.H., Lee, J.J. Antiinflammatory constituents of celastrus orbiculatus inhibit the NF-B activation and NO production. Journal of Natural Products, 2002;65: 89–91.

[8] Li, H., Jia, Y.F., Pan, Y., Pan, D.J., Li, D., Zhang, L.X. Effect of tripterine on collagen induced arthritis in rats. Acta PharmacologicaSinica 1997;18: 270–273.

[9] Li, H., Zhang, Y.Y., Huang, X.Y., Sun, Y.N., Jia, Y.F., Li, D.Beneficial effect of tripterine on systemic lupus erythematosus induced by active chromatin in BALB/c mice. European Journal of Pharmacology 2005;512: 231–237

[10] Huang, F.C., Chan,W.K., Moriarty, K.J., Zhang, D.C., Chang, M.N., He,W., Yu, K.T. Zilberstein, A., Novel cytokine release inhibitors. Part I Triterpenes. Bioorganic & Medicinal Chemistry Letters 1998;8: 1883–1886.

[11] Zhang, L.X., Yu, F.K., Zheng, Q.Y., Fang, Z., Pan, D.J. Immunosuppressive and anti inflammatory activities of tripterine. Acta Pharmaceutica Sinica 1990;25: 573–577.

[12] Noguchi, M., Kimoto, A., Kobayashi, S., Yoshino, T., Miyata, K., Sasamata, M. Effect of celecoxib, a cyclooxygenase-2 inhibitor, on the pathophysiology of adjuvant arthritis in rat. European Journal of Pharmacology 2005;513: 229–235.

[13] Simoes, S.I., Delgadoa, T.C., Lopes, R.M., Jesus, S., Ferreira, A.A., Morais, J.A., Cruz, M.E.M., Corvo, M.L., Martins, M.B.F.Developments in the rat adjuvant arthritis model and its use in therapeutic evaluation of novel non-invasive treatment by SOD in transfersomes. Journal of Controlled Release 2005;103:419–434.

[14] Smith, R.J. Therapies for rheumatoid arthritis: hope springs eternal. Drug Discovery Today 2005;10:1598–1606

[15] Basaran, A . A.; Yu, T.W.; Plewa, M.J.; Anderson, D. 1996. An investigation of some Turkish herbal medicines in Salmonella typhimurium and the Comet assay in human lymphocytes. Tetratog. Carcinog. Mutagen., 16, 125-138.