

A Phytotherapeutic Formulation for Allergic Rhinitis in Autistic Children

ABSTRACT

Aim: In this study the efficacy of a nasal spray based on extract of lemon pulp for treatment of allergic rhinitis in autistic subjects by nasal cytology was evaluated. The extract of lemon pulp was obtained by means of an innovative technology of solid-liquid extraction using Extractor Naviglio or Rapid Solid-Liquid Dynamic Extractor, that extracts vegetable matrices of each species, by using different types of solvents. Due to pressure-depressure action, by using water as a solvent it is possible to extract both hydrophilic and lipophilic substances contained in vegetables in heterogeneous solution.

Objectives: In our research was used a nasal spray based on extract of lemon pulp in for treatment of allergic rhinitis in autistic subjects, for which there are legal limitations for traditional treatments available, that require the use of cortisone, antihistamines, leukotriene modifiers, in relation to the possible harmful side effects linked to long term therapy. Moreover, this nasal spray can also be administered to very young children, being devoid of alcohol

Methodology: The efficacy of the product has been tested on 20 patients aged between three and eight years (10 males and 10 females, mean age 5.5 years) The cytology was performed by exfoliative technique, with a sterile swab soaked in sterile saline solution and then crawled on the middle part of the inferior turbinate. The collected sample was stained with the method of May-Giemsa Grumwald to highlight the morphological changes of the nasal epithelium before and after therapy based on nasal-spray.

Results: Experimental data demonstrated that the anti-inflammatory effect of the lemon based nasal spray is a safe medical device as an aid in the reduction of inflammatory cells observed on rinocitogram

Conclusion: The lemon based nasal containing extract of lemon pulp can be used in all autistic patients, even in children, given the absence of alcohol.

Keywords: Autism; Allergic rhinitis; Nasal cytology; Nasal spray; Lemon pulp

1. INTRODUCTION

Autism, originally called Kanner's syndrome, is considered by the international scientific community a disorder that affects brain function, the person suffering from this disease shows a marked decrease in social integration and communication. More precisely, given the variety of symptoms and the clinical complexity of syndrome, has recently been proposed the definition of Autism Spectrum Disorders (ASD). Currently are still unknown causes of this event, but there are many theories that currently linking autism to brain damage resulting in damage neurocognitive early. The exposure to toxic metals, environmental pollution and poor dietary habits may interact with genetic factors predisposing characterized by immune dysfunction, gastrointestinal abnormalities, becoming etiological factors.

Previous clinical and genetic studies have suggested autism spectrum disorders (ASDs) is associated with immunological abnormalities involving cytokines, immunoglobulins, inflammation, and cellular immunity, but epidemiological reports are still limited [1]. In a recent epidemiological study, a total of 1596 patients with ASDs were identified, and were found to have a significantly higher prevalence of allergic and autoimmune diseases than the control group. In particular, patients with ASDs had increased risks of allergic rhinitis (OR=1.70, 95% CI=1.51-1.91)[2]. These results supported the association between ASDs and allergic diseases, and autoimmune comorbidities (type 1 diabetes and Crohn's disease), though further studies are required to elucidate the possible underlying mechanisms and roles of allergy immunity and autoimmunity in the etiology of ASDs.

In some studies a correlation has been shown between ASD mastocytosis and eosinophilia [3,4,5] pathological form complex that involves both the activation and proliferation of cutaneous mast cells, with the appearance of nettle-rash pigmentosa, both of other organs leading to skin reactions, allergies food, rhinitis, asthma, often in the absence of positive skin test [6,7]. Mast cells and eosinophils are not only determinants for allergic reactions, but also for inflammations, being involved in the permeability of the tissue membranes [8,9,10]. Very important is the search for therapeutic methods are able to inhibit their proliferation. The flavonoids are a group of plant pigments responsible for much of the color of many fruits, vegetables and flowers with antioxidant activity. Under this name are collected 5000 compounds classified into subclasses, depending on their chemical structure. The health effects of flavonoids have been analyzed in recent years and relate to the antioxidant activity, antiinflammatory, antiallergy, antiviral and antitumor activity. The effect of flavonoids seems to be mainly preventive. In particular, some natural flavonoids such as quercetin and luteolin seem to reduce the release of inflammatory molecules, like histamine and kinins from mast cells. Quercetin can reduce oxidative stress in autistic subjects with the decrease in the level of lipid hydro-peroxides and antioxidant enzymes [11]. Luteolin inhibits the production of microglia, reducing the inflammatory action of glial cells [12,13]; inhibits the release of cytokines; has neuroprotective action, for which may be useful in the treatment of neuroinflammatory diseases, alone or as adjuvant other therapeutic approaches. Flavonoids, however, and in particular the luteolin, are lipophilic substances and are poorly absorbed after oral administration, being metabolized by the liver. Acute infections of the upper respiratory tract are common among autistic children as rhynosisinusitis sinusitis, pharyngitis/tonsillitis, ear infections, laryngitis, rhinitis and allergic asthma. Asthma is one of the most common chronic diseases and a major cause of morbidity in children worldwide. The symptoms of asthma in children include recurrent episodes of wheezing, dry cough, chest tightness with inflammation and airway obstruction: triggering factors for the disease are atopy, allergens, infections, obesity, smoking.

2. NASAL CYTOLOGY

Nasal cytology is of remarkable importance in the study of rhino-sinus diseases, especially the Vasomotor Rhinitis (VMR), as it represents a valuable means of differential diagnosis between allergic/non-allergic diseases and bacterial/viral infections. It is a popular and proven method, considering that it dates back to 1889, when first Gollash [15] identified the numerous eosinophils in the nasal secretion from an asthmatic patient and attributed their presence a key role in the pathogenesis of asthma. The nasal cytodiagnosis was recently encouraged by the study of Charles Eyermann [16], who identified the eosinophils in the nasal exudate of allergic patients and underlined its diagnostic importance. Since then, lots of researchers have focused their attention on cytology and particularly on the presence of different types of inflammatory cells in nasal diseases.[17,18]. Different factors have contributed to the increased interest in cytological study of the nasal mucosa, making this procedure more widespread: the sampling is easy to perform and minimally invasive, allowing the examination to be repeated, as often required in the follow-up visits in the case of vasomotor disorders and for monitoring the effectiveness of some treatments. Some studies have proved that the rhinocytogram of patients with allergies varies according to the topical nasal steroid

88 treatment. Some authors have shown that fluticasone dipropionate and beclomethasone
89 dipropionate are able to effectively control the symptoms of perennial and seasonal
90 allergic and vasomotor rhinopathy and to induce cytological changes with a significant
91 reduction in the number of eosinophils and basophils in the nasal mucosa [19,20,21]
92 showed that the anti inflammatory effect of topical corticosteroid is doubtlessly proven by
93 the reduction in the immune-inflammatory components observed on the rhinocytogram.
94 The cortisone therapy, despite being effective in most cases, presents disadvantages
95 related to side effects after prolonged use; it is not tolerated by allergic individuals; it may
96 not be used during pregnancy and lactation; finally, it may not be used by children under
97 the age of twelve.

98 99 **3. LEMON**

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101 The lemon belongs to the family Rutaceae, which also includes oranges, mandarins,
102 bergamot, cedar, grapefruit. The main cultivars of lemons are: Femminello, Monachello
103 and Interdonato. In particular, the femminello Sorrentino, also known as lemon "Oval of
104 Sorrento" and "lemon of Massa" has medium-large dimensions (each lemon weighs no
105 less than 85 grams), a pulp straw yellow in color with a highly acidic juice and is rich in
106 vitamin C. Lemon is known for its therapeutic properties for several generations, it helps
107 to strengthen the immune system and cleanses the digestive tract. Moreover, it is not
108 only a blood purifier but also helps the body to fight disease. The lemon juice, in
109 particular, is very useful in the treatment of kidney stones, in the treatment of heart
110 attacks and in the reduction of body temperature. The health benefits of lemon are due
111 to many elements contained in it, such as vitamin C, vitamin B, phosphorus, proteins and
112 carbohydrates as well. Lemon is a fruit that contains flavonoids, which, in turn, contain
113 elements antioxidants, has an anti-inflammatory effect, acts as a natural antihistamine
114 and has anti-cancer properties. Helps to prevent diabetes, constipation, hypertension, is
115 helpful in skin care, in the treatment of fever, in hair care, on the occasion of dental
116 therapies, in case of indigestion and many other health problems [22]. Some studies also
117 show that lemon juice or lemonade are able to cure kidney stones by forming urinary
118 citrate, which prevents the formation of crystals [23] .
119 The aim of this study was to examine the behavior of the nasal membrane in autistic
120 subjects before and after treatment with nasal spray made of pulp extract of lemon.
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122 123 **4. MATERIAL AND METHODS**

124 **Instrumentation and chemicals.** Naviglio Extractor 500 mL model (Atlas Filtri
125 Engineering, Padua, Italy); Microscope (Nikon Instruments S.p.a., Florence, Italy); May-
126 Grünwald- Giemsa Reagent (Carlo Erba, Milano, Italy).
127 Lemon extract 1% titrated with citric acid 6%; pure aloe juice, essential oil of ravsara,
128 soluble Propoli WSEP-70®, Essential oil of Niaouly (Intermedia Synergie s.r.l.,
129 Cernobbio, Como, Italy) were used in the preparation of nasal spray.
130 **Procedure.** For the preparation of the extract of lemon pulp was used the Naviglio
131 extractor, demineralized water and 2 kg of lemons (12 lemons) following the procedure
132 previously reported [24]. Lemon extract showed a pH between 3 and 3.5 and a citric
133 acid content ranging between 6% to 7% (w/w); the juice of Aloe barbadensis Miller,
134 Propoli WSEP-70® and small quantities of Ravensara Niaouly essential oil were added
135 to obtain a nasal spray with no preservatives and no alcohol, suitable even for kids.
136 **Recruitment of patients and therapy.** Twenty patients were selected, relating to AIAS
137 (Italian Association to Spastic Assistance) structure of Afragola (Naples, Italy), both male
138 and female, mean age 5.5 years (min. 3, max 8 years), showing autistic spectrum not in
139 drug treatment. All parents accepted the administration of the spray and protocol of
140 sampling.
141 For all subjects after completion of the formalities examination was performed amnesic
142 and objective in the presence of the parents who helped to define the clinical picture of
143 the subject, which has been designated a number from 0 (no symptoms) to 3 (presence
144 of any allergic symptoms); during treatment was necessary in addition to the presence of

the parents that qualified personnel to prevent panic attacks or bouts of hyperactivity, very difficult to manage.

In the study particular attention has been paid to sampling times and cytological processing. The cytological sampling was performed by exfoliative technique, with a sterile swab soaked in sterile saline and then crawled on the middle part of the inferior turbinate. The material thus collected was then transferred onto a glass slide for microscopy by stretching thoroughly for stratify them in a thin layer and has subsequently been subjected to staining by following the method of May-Grunwald-Giemsa, preferred by us as able to color all the cellular components normally present in normal conditions or immune inflammation.

Observation with an optical microscope, Nikon Eclipse 200, into an immersion 1000x was made and then we proceeded to a reading for fifty fields, examining the entire surface of the slide to find cell phones with greater interest for the purposes of diagnosis (neutrophils, eosinophils, lymphocytes, mast cells). At the same time we proceeded to the observation of the cells of the nasal epithelium.

It was first performed cytology based upon the inclusion of patients in the study (Figures 1-6). The winds autistic subjects were divided into two groups of ten.: Group A who received the lemon based nasal spray: Lemon Extract 1% citric acid titrated to 6%, pure aloe vera juice, essential oil Ravensara, Propolis-soluble WSEP 70®, essential Oil Niaouli (two puffs - equal to 0.14 ml -3 times daily); Group B which has been administered an equal amount of saline. (two puffs - equal to 0.14 ml-3 times day).

Figgs 1.2.3. relating to rhinocytograms of patients in group A, before treatment with the nasal spray with a score of 2, 2, 3 In Fig.1, Fig.3 can see the cells eosinophils by the blue arrow; in Fig.2 can see mast cells by the red arrow

It was first carried out a cytology based upon the inclusion of patients in the study, which are reported as some Rhinocytograms (Figures 1-6). Figures relating to rhinocytograms of patients in group A, with a score of 2, 2, 3 before treatment with the nasal spray you can see the cells eosinophils shown in Figure 1 and Figure 3 by the blue arrow, mast cells by the red arrow in Fig. 3 indicate, with large areas of eosinophil degranulation.

Figgs 4.5.6. relating to rhinocytograms of patients in group B, before treatment with saline solution with a score of 2, 2, 3 In Fig.4, Fig. 5 can see the ast cells and eosinophils; in Fig.6 as mast cells are also present neutrophils indicated by the black arrow and a large area of eosinophil degranulation

In the figures relating to rhinocytograms of patients in group B with 2,2,3 score before treatment with saline solution it is possible to note mast cells and eosinophils in Figure 4 and Figure 5, while in Figure 6 as well as mast cells are also present neutrophils indicated by the black arrow and a large area of eosinophil degranulation. After 10 days of therapy with nasal spray has proceeded to nasal cytology control; subsequently we proceeded to rhinocytological control in all cases after 30 days of therapy. (Figures 7-12) Wilcoxon-Two-Sample Test (t approximation) for the statistical evaluation of the experimental results was used, with an evaluation of statistically significant $p < 0.05$.

Figgs 7.8.9 relating to rhinocytograms of patients in group A after topical administration of the spray lemon Can see the almost complete disappearance of eosinophils and mast cells, with the persistence of some neutrophil and eosinophil granule

Figgs 10.11.12 relating to rhinocytograms of patients in group B after treatment with saline solution: no evidence of significative changes was detected

5. RESULTS

The evaluation of the rhinocytogram in autistic subjects, highlighted a rich neutrophilic component, with a discreet but constant representation of eosinophils and mast cells, in both group before the therapy (See Figures 1-3). In all group A examined subjects, in the first ten days, was reported to be an improvement examination. The topical administration of the spray lemon based showed at the end of therapy the almost complete disappearance of eosinophils and mast cells, with the persistence of some neutrophil and eosinophil granules and metachromatic very rare in the treatment period regard the group A, while in the group B : (Table 1). It is therefore essential during treatment, constant toilet nasal washes or showers with saline solutions, in order to remove all the conditions conducive to the unfolding of the allergic reaction. Sometimes, for the severity and subjective symptomatology it is necessary to increase the dose of the spray lemon-based, without however observing side effects.

TAB. 1 Total progress of inflammatory cells for both group before and after therapy.

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The analysis of rhinocytograms is in perfect harmony with the clinical improvement of autistic patients reported by the parents. In fact, to an improvement of the overall symptom, has always corresponded to a reduction of the inflammatory cells of the nasal secretion. The improvement of the rhinocytological framework is given by control mechanisms due to the synergistic action of the natural substances present in the spray and still under study. Although the small cases to draw conclusive remarks, the positive results to the likelihood that the spray has definitely anti-inflammatory action.

6. DISCUSSION AND CONCLUSION

Different studies witness daily the efficacy of lemon extract on the nasal mucociliary clearance and to the properties of water-soluble flavonoids on venous micro-circulation [24,25,26]. The trial of nasal spray under study, in addition to following results concerning the cytology showed immediate effects, such as the liberation of the nose and leading to a better breathing. Since the first applications of the product showed a generous elimination of fluid that helps to remove deposits of mucus and irritants, promoting accurate nasal hygiene. The essential oils present exert a cooling effect contributing sanitizing action. Natural substances present in the spray exhibited anti-inflammatory properties undoubtedly, therefore they are indicated in all those diseases, where the use of corticosteroids may be contraindicated.

There are accounts in literature that lemon juice is amongst the most powerful natural antiseptic and bactericide; it is beneficial in ear infections and in colds, and has a certain efficacy in treating inflammations of the throat, mouth ulcers, gingivitis and inflammations of the tongue.

Lemon juice contains hesperedin, eriodictyol, and diosmin [25,26]. The pharmacological effects of flavonoids were first observed in 1935 by Szent-Gyorgyi,[27] who separated from the lemon peel a substance capable of decreasing capillary permeability and of increasing vascular resistance, a substance called citrina, which was later discovered to be made of two flavonoids, hesperidin and eriodictyol.

Aloe juice has an anti-allergenic and anti-allergic effect, proving to be highly efficient in most cases; moreover, the effect of Aloe tincture has long been known in nasal-oro pharyngeal infections [28]. The complex acemanane sugar together with the bradykinin present in the aloe have a reinforcing and modulating effect on the defensive and anti-inflammatory system, as confirmed by clinical studies that show its antioxidant, immunostimulant, anti-inflammatory, anti-allergic and purifying effects [29]. The carboxypeptidase that is also present in the aloe, instead, acts on tissue inflammation and indirectly alleviates the pain.

The essential oil of Niaoulj represents an effective protective agent in the treatment of infections to the breathing tracts, because its vapors have bactericidal, immuno stimulant, hyperemizing, mucolytic, and balsamic properties [30]. Hence, the Niaoulj can be used as a valid remedy to sustain the functions of the respiratory apparatus in the event of: rhinitis, pharyngitis, laringo-tracheitis, bronchitis, and pneumonia. Moreover,

thanks to its anti-spasmodic properties, the vegetable extract of Niaoulj finds application as a remedy to sustain the organic functions in the presence of spasmodic coughs [31]. Thanks to its great anti-bacterial, anti-viral, and expectorant properties, the essential oil of Ravensara represents an excellent remedy in infection to the breathing tracts. It can be used as a valid remedy to sustain the functions of the respiratory apparatus in the event of sinusitis, rhino-pharyngitis, bronchitis, coughs, and whooping coughs [32]. The propolis WSEP-70® standardized at 10% (w/w) in quercetin and 75% (w/w) in total polyphenols expressed in galangin is an extract of water-soluble propolis, with the capacity of favoring the natural defense of the organism against inflammatory disturbances to the nasal and oropharyngeal cavities[33]. It displays a high anti oxidizing and protective power for the mucosa, due to the high bio-availability of the active principles on a level of biological fluids, and to the high absorption of active components on a level of oropharyngeal mucosa and on the first gastro-esophageal tract, compared to those of traditional propolis[34]. Upon the first observation, the rhinopathic subjects displayed the typical symptoms of the allergy, that is: nasal obstruction, rhinorrhea, sneezing, more or less accentuated. Hypertrophic turbinates of a bruised-pale coloring appear with the rhinoscopy. In Tables 2 and 3 are reported the values of the scores before and after treatment relative to the group A and B. The patients subjected to the treatment, from a subjective-overall symptomatology point of view, displayed before the therapy the following scores: group A: 70 % a score 3, 20% a score 2, 10% score 1. Scores of group B were: 70 % a score 3, 20% a score 2, 10% score 1. After therapy the scores of group A were: 100% score 0; while the score of group B were: 65% score 3, 20% score 2 , 15% score 1. (Table 2 and 3). The evaluation of the initial group A rhinocytograms documented a rich neutrophilic component, with a discreet but constant representation of eosinophils and mast cells, in nine cases rare lymphocytes. In all subjects examined, in the first ten rounds, it was detected a clear regression of symptoms with adequate standards of objectivity and nasal symptoms individually; only in two cases it was necessary to increase the number of daily doses, because of allergic symptoms and persistent. At the microscopic observation, it was observed an absence of neutrophils and lymphocytes, with a reduction of more than 50% of eosinophils and mast cells. In some cases in rare degranulations eosinophilic and mast cells have been observed.

Tab. 2. Group A score before and after the treatment. **Table 3.** Group B score before and after the treatment.

At the end of therapy, all subjects of group A, have reported a symptomatology individual equal to 0 (no symptoms). This has been confirmed by an examination local objective, which documented a clear improvement of the mucosa, both by rhinocytograms that has detected a reduction of mast cells (average 20.26 to 2.18; standard dev. 19.56 to 3.17) of eosinophils (mean 13.6 to 2.5; standard dev. 16.4-6.38) neutrophils (average 37 to 1.74; standard dev. from 55.21 to 5.15) and lymphocytes (average from 5.32. to 0.88, standard dev 11.45 to 2.95) ($p < 0.01$). Subjects in Group B did not show substantial improvements or cytological or symptom ($p < 0.01$). Analysis of the results of our study follows that of people with autism, there is a greater susceptibility to colds phones and that the nasal cytology, a method we use is undoubtedly valid, as well as being well tolerated and easy to perform [14-18] .

The shapes studied have documented a cytologic pattern represented by a significant proportion of neutrophils, eosinophils and mast cells pathognomonic of cellular forms [35-39]. Lymphocytes are probably related to previous viral infections.

The main aim of our study was the assessment of the variation in cellularity nasal, in particular of those cells known for their involvement in the determination of the framework and of allergic complications in the course of local therapy with a nasal spray lemon based. A placebo effect was verified by means of the control group.

The topical administration of the spray lemon based showed the almost complete disappearance of eosinophils and mast cells, with the persistence of some neutrophil or lymphocyte and rare eosinophils and metachromatic granules. It is therefore essential during treatment, a constant of the toilet pit with nasal washes or showers of saline solutions, in order to remove all the conditions conducive to the unfolding of the allergic reaction. Sometimes, due to the severity and subjective symptoms, it is necessary to increase the dose of the spray made with lemon, without side effects. In addition, you need a constant power output to achieve optimal treatment.

The analysis of rhinocytograms are in perfect harmony with the clinical improvement. In fact, to an improvement of subjective symptoms, has always corresponded to a reduction of the inflammatory cells of the nasal secretion. The improvement of the rhinocytologic framework is given by control mechanisms due to the synergistic action of the natural substances present in the spray and still under study by the botanical. Our survey, however, is still small to draw general conclusions.

We can affirm that the spray can be used in all those autistic children, in which the traditional therapy is contraindicated, due to the absence of alcohol-based substances. Moreover, we can argue that the nasal cytology may be a useful method and objective to assess, not only the phases of a clinical pathology rhinitis, but also for monitoring the effects of therapy on the component of inflammatory cells, the reduction of which is a guarantee of effectiveness therapeutic

COMPETING INTERESTS

All authors can confirm that there is no conflict of interest, financially or otherwise

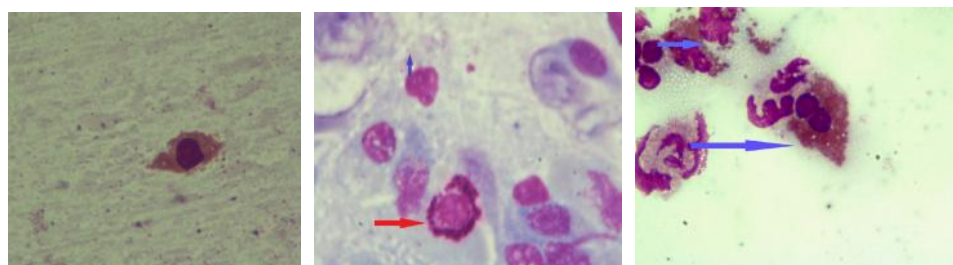
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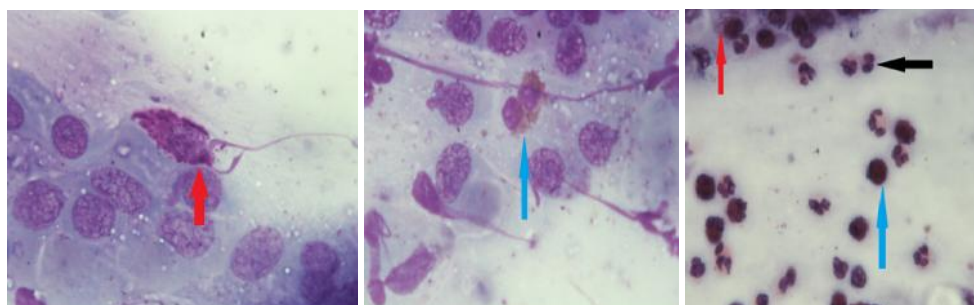
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FIGURES and TABLES (First part)



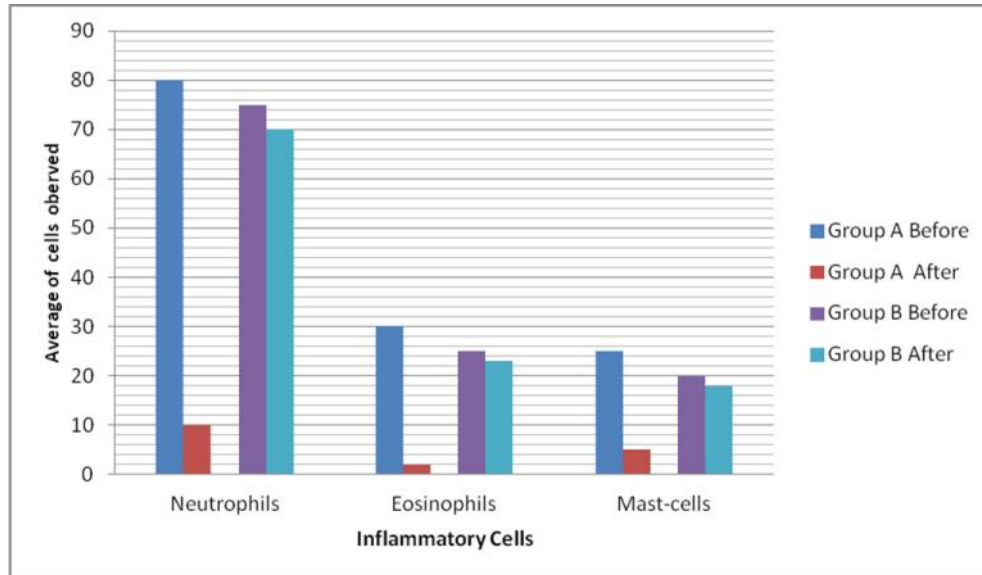
Figs 1.2.3.



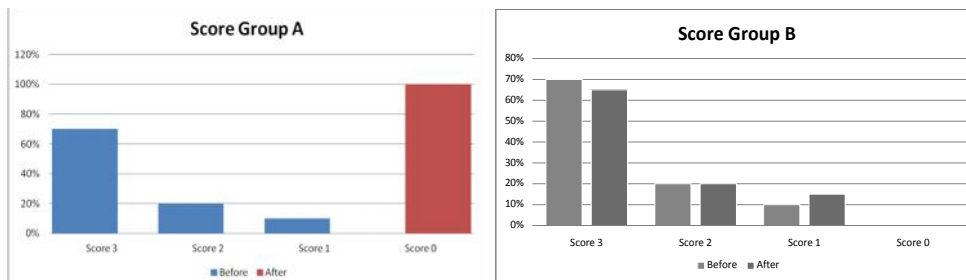
Figs 4.5.6.

FIGURES and TABLES (Second Part)

TAB. 1 Total progress of inflammatory cells for both group before and after therapy.



Tab. 2. Group A score before and after the treatment. **Table 3.** Group B score before and after the treatment.



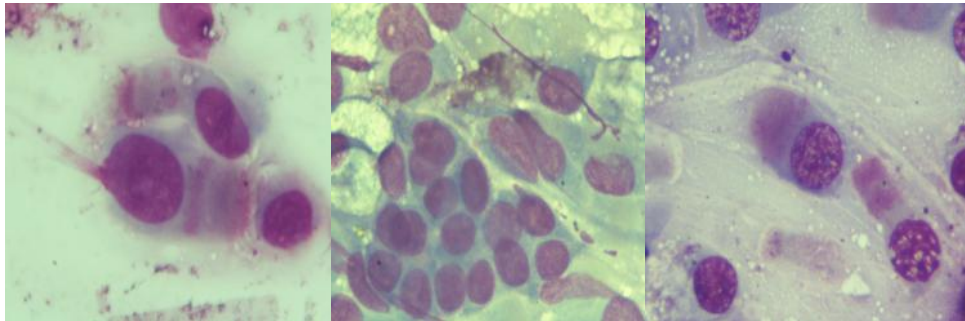
FIGURES and TABLES (Thirh part)

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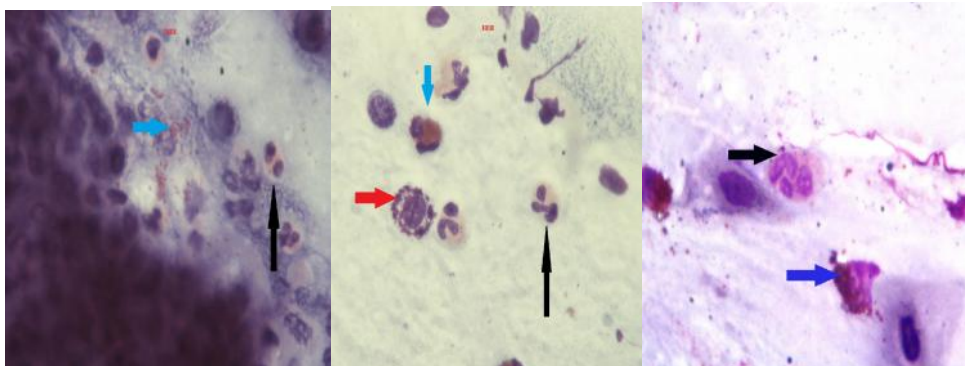


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Figs 7.8.9



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Figs 10.11.12