Title: Comparative clinical study of effectiveness of "MEKRITEN" in patients with chronic suppurative otitis

ÖZ

Giriş. Kronik süpüratif otitis media (KSOM), orta kulakta bakteri enfeksiyonu bulunan ciddi bir hastalıktır. Dünya çapında, gelişmiş ve gelişmekte olan ülkelerde yaşayan nüfusun 1% - % 46'sında kronik süpüratif otitis media muzdarip, yaklaşık 65-330 milyon insan, bunların % 60'ında önemli işitme kaybı var. Özbekistan Cumhuriyeti'nde klinik kullanım için uyuşturucuya tavsiyede bulunma olasılığını belirlemek için "Sarımsak Özü sıvısı" ilacının yara iyileştirme etkinliğinin ve tolere edilebilirliğinin incelenmesi.

Yöntemler. Çalışma ilacını alan ana grup 30 hasta, karşılaştırma ilaç alan hasta grubunda 20 hasta bulunmamaktadir. Ana grubun (30 kişilik) hastaları, Taşkent İlaç Enstitüsü tarafından geliştirilen "Sarımsak Özü sıvısı" olarak dış kulak yolu kanalında günde 2 damla 10 gün süreyle atandı. Karşılaştırma grubundaki şikayet edenler arasında (20 kişi) benzer şekilde diğer ilaçları (% 0.25 solüsyon Levomycetin) aldı. Hastalar, ağrı, sekresyon (puan olarak) gibi şikayetlerin ciddiyetini belirlemek için dikkatli bir şekilde incelendi ve gözlemlendi; Ayrıca gözlemciler biyokimyasal (ALT, AST, bilirubin), enstrümantal (karaciğer ultrasonu) ve ekineokoksik kapsüllerin fibröz dokusunun biyopsi analizlerini inceledi.

Sonuçlar. "MEKRITEN" puanlarındaki tolere edilebilirlik ve etkililiğin ortalama derecelendirmesi, 4.97 puanlık taşınabilirlik ve 4.77 puanlık etkililiği, sırasıyla 4.8 ve 3.35 puanlık Levomycetin çözeltisi için % 0.25'e düşürdü???. "MEKRITEN" alırken hiçbir yan etki ve alerjik reaksiyon gözlenmedi. Bir hastanın ilacın kesilmesini veya bu hastadaki ilacın etkisizliğini gerektiren advers etkilere sahip olması veya ilacı almayı kaçırması veya hastanın çalışmaya devam etmesini reddetmesi durumunda, bazı hastalar için araştırma durdulabilir. Yukarıdaki nedenlerden hiçbiri yoktu; Bu nedenle, herhangi bir hastada çalışma durdurulmadı.

Netice. Kronik süpüratif otitis media hastalarında "MEKRITEN" (yağlı sarımsak özütü) ile yapılan lokal terapi, % 0.25'lik Levomisetin solüsyonunun lokal uygulanmasından daha etkilidir. Kronik süpüratif otitis media tedavisinde "MEKRITEN" ile lokal terapi, otorrinin daha çabuk kesilmesine ve daha az advers ilaç reaksiyonuna neden olur.

Anahtar Kelimeler: MEKRITEN; Levomisetin; Kronik süpüratif otitis media; Sarımsak özütü sıvı.

ABSTRACT

Background. Chronic suppurative otitis media (CSOM) is a serious disease with the presence of bacterial infection in the middle ear. Worldwide, from 1% to 46% of the population live in the developed and developing countries suffer from chronic suppurative otitis media, it is about 65-

330 million people, 60% of them have significant hearing loss. The study of wound healing efficacy and tolerability of the drug "Garlic Extract liquid" is dedicated to identify the possibility of issuing recommendations to the drug for clinical use in the Republic of Uzbekistan.

Methods. The main group of patients, who received the study-drug, consisted of 30 patients; in the group of patients who receiving the comparison drug, there were 20 patients. Patients of the main group (30 persons) were appointed "Garlic Extract liquid" which was developed by the Tashkent Pharmaceutical Institute, and it was used to 2 drops 2 times a day for 10 days in the external auditory canal. Patients in the comparison group (20 people), took other drugs (0.25% solution of Levomycetin) in a similar way. Patients were inspected and observed carefully for determining severity of the complaints such as pain, secretion (in points); and checked clinical analyses: General blood analysis, urine etc. In addition, observers studied biochemical (ALT, AST, bilirubin), instrumental (ultrasound of the liver) and special (biopsy of fibrous tissue of echinococcal capsules) analyses.

Results. The average rating of the tolerability and effectiveness in the points for "MEKRITEN" made portability of 4.97 points, and the effectiveness was 4.77 points, for 0.25% Levomycetin solution they were 4.8 and 3.35 points, respectively. While receiving "MEKRITEN", no side effects and allergic reactions were observed. The research for a particular patient can be stopped if patient has adverse reactions requiring discontinuation of the drug, or the ineffectiveness of the drug in this patient, or if he missed taking the drug, or refusal of the patient from further participation in the study. There was no any of the above reasons; thus, the study was not stopped in any of the patients.

Conclusion. Local therapy with the drug "MEKRITEN" (Garlic Extract liquid) in patients with chronic suppurative otitis media is more effective than local application of 0.25% Levomycetin solution. Local therapy with "MEKRITEN" in the treatment of chronic suppurative otitis media leads to faster termination of otorrhoea and is accompanied by fewer adverse drug reactions.

Keywords: MEKRITEN; Levomycetin; Chronic suppurative otitis media; Garlic Extract liquid.

INTRODUCTION

Chronic suppurative otitis media (CSOM) is a serious disease with the presence of bacterial infection and perforated tympanic membrane with persistent drainage from the middle ear. It is a major cause of acquired hearing impairment in children, especially in developing countries. Most approaches to treatment have been unsatisfactory or are very expensive and difficult; for example, parenteral aminoglycosides require long hospitalization and are potentially ototoxic [1]. The pathological process in chronic purulent otitis media leads to destruction of bone

structures of the middle ear and cause to hearing loss. Despite the use of antibacterial therapy, chronic suppurative otitis media remains the main cause of hearing loss [2].

Chronic suppurative otitis media is one of the most urgent problems of otorhinolaryngology, because it is a significant part of the whole pathology of ear, nose and throat (ENT) organs; and ranks second in the structure of otorhinolaryngology morbidity. Prevalence surveys, which different widely in disease definition, sampling methods, and methodological quality, show that the global burden of illness from CSOM involves 65-330 million individuals with draining ears, from 1% to 46% of population, who live in developed and developing countries, suffer from chronic suppurative otitis media, 60% of whom suffer important hearing impairment. Annually, 31 million new CSOM cases are registered in the world, 22.6% of whom are diagnosed in children who are under 5 years old. In 30.82 cases per 10,000 population, the disease is accompanied by hearing loss. In the world every year from complications of chronic suppurative otitis media 28000 people die (mostly from intracranial complications) [2].

Among all chronic diseases of ear, nose, throat (ENT) organs, chronic suppurative otitis media is the most frequent pathology (up to 48.8%). Among patients with ENT pathology, who are assisted in the ENT departments, 5.7–7% suffer of CSOM. CSOM with frequent exacerbations is the cause of otogenny complications that currently appear in 3.2% patients: in 1.97% intracranial is observed (meningitis, brain abscess, etc.) and in 1,35% - extracranial (subperiosteal abscess, labyrinthitis, etc.) complications. Mortality from CSOM is 16-30%. One of the reasons of development of destruction in the middle ear is cholesteatoma, which is revealed to 24-63% patients with CSOM at any location of the perforation of the eardrum.

Both acute and chronic suppurative otitis media are still one of the most common diseases of ENT organs and ranged from 5.1 to 58% of cases, and from 8.6 to 37% of cases are the cause of high levels of hearing loss [3, 4]. Annually, 31 million new cases of chronic suppurative otitis media (CSOM) are registered in the world, 22.6% of them are diagnosed in children younger than 5 years [5].

That is why the issues of early diagnosis, choice of treatment and characteristics of patients with CSOM are still relevant. However, the solution of these problems is closely connected with the study of various aspects of the etiopathogenesis of the disease, including general and local immunological reactivity, the state of the antioxidant system of the organism, i.e. the background, where there is probably a pathological process.

In connection with the above, the efficacy of 'Oil extract of garlic' was developed by TashPharm, and was investigated and prepared Garlic Extract liquid. In preclinical studies, the drug showed high efficacy in experimental models.

Purpose: the study of wound healing efficacy and tolerability of the drug "Garlic Extract liquid" developed by the Tashkent Pharmaceutical Institute, Uzbekistan, to identify the possibility of issuing recommendations to the drug for clinical use in the Republic of Uzbekistan.

MATERIALS AND METHODS

This study was open, full designed and performed on two parallel groups. The main group of patients, who received new drug, consisted of 30 patients. The next group of patients, who received the comparison drug, were 20 patients. The groups were matched by sex, age and diagnosis. Patients receiving outpatient treatment, of both sexes, aged over 18 years, and who gave written informed consent for participation in the research, and those who underwent surgery in the middle ear (myringoplasty) [2].

The criteria for exclusion in the research

- The age of the patients up to 18;
- Pregnancy;
- Lactation;
- The presence of hypersensitivity to the drug component;
- Participation of patient in other clinical trials within last 30 days;
- No written informed consent of patient for participation in a clinical research;
- Contraindications to the use of the drug.

Patient details

The first group included 30 patients, which was aged 34±12.33; 13 patients were women and other 17 were men; 8 patients were diagnosed with right-sided CSOM, in 11 cases were hospitalized with left CSOM, and other 11 cases were with bilateral CSOM.

Second group were consisted of 20 patients and average age was 35±13.42. According to a gender difference, 12 patients were female and 8 patients were male. 10 patients were diagnosed right-sided CSOM, 6 patients were with left CSOM, and 4 patients were treated with bilateral CSOM.

The scheme of drugs appointment

Patients of the main group (30 persons) were appointed "Garlic Extract liquid", which was developed by the Tashkent Pharmaceutical Institute, Uzbekistan. The medication was given 2 drops 2 times a day during 10 days in the external auditory canal. Patients in the comparison group (20 people), took other drugs (0.25% Levomycetin solution) in a similar way. Complementary therapies were not performed simultaneously**.** Other drugs with a similar action

were excluded. Medications and other drugs that are compatible with the drug, as well as the necessary physical therapy were used for necessary treatment of the underlying disease.

The overall schedule of the research

- After the initial examination of patients corresponding to inclusion criteria to obtain their written informed consent to participate in the study, they are provided with the information about the experimental drug "Garlic Extract liquid", information about doses, schemes, routes of administration and period of treatment.
- In the case of obtaining the patient's written consent for participation in the research, he was administered the study drug or the comparison drug.
- The starting point of the patient's participation in the study: the first day of receiving study drug or the comparison drug.
- Treatment was described in all patients included in the study.
- Any treatment related to concomitant diseases was registered in the medical history and individual registration form.

The receipt, use, storage of the investigatory drugs and procedures of verification of compliance of the studied patients' prescription

Drugs with registration of the act of transfer and acceptance is transmitted by the Customer to the responsible contractor (or Department head. pharmacy) and must be kept indoors, access to which only the responsible officer or head pharmacy apart from other has prescribed drugs.

A responsible person for issuing drugs to patients is needed to be assigned.

To register for the issuance of patients' medications, the forms of the issuance of the test drug and comparison drug, with signatures of patients, certifying each of the preparation should be filled.

The start date of the testing for each patient – the date of the first dose of study drug or the comparison drug taking. Date of completion of testing for each patient, the last date of the study drug or the comparison drug taking. In the medical history, medical card it must be recorded that the patient voluntarily agreed to take the testing drug as follows:

The patient was provided with the informed consent form for participation in a clinical trial. The patient had enough time for decision-making. The patient signed the informed consent. Procedures for verification of patient compliance of doctor orders are governed by the internal regulations of hospital, where the study is conducted. When there is a break in the procedure of taking the drug, the patient is excluded from the study [3].

Responsible person will not allow the use of the test drug and comparison drug for any other purpose except that specified in the protocol of a clinical trial.

After the completion of the study, a report on the use of the test drug and comparison drug is made by form 2 of Appendix №2 to the order of Ministry of Health (MH) of the Republic of Uzbekistan (RUz) №334 dated from 25.07.2001.

Examination

Patients were inspected and observed carefully for determining severity of the complaints such as pain, secretion (in points); and checked clinical analyses: General blood analysis, urine etc. In addition, observers studied biochemical (ALT, AST, bilirubin), instrumental (ultrasound of the liver) and special (biopsy of fibrous tissue of echinococcal capsules) analyses.

Criteria for evaluating the effectiveness of the study drug

The list of performance indicators:

- the complete disappearance of the inflammatory process and pain
- normalization of laboratory and instrumental studies.

Evaluation of the effectiveness of an investigational drug will be conducted by the researcher on the basis of the above criteria in points according to the following scale:

3 points	High efficiency	A marked disappearance of complaints: no pain and secretions (points 0-2), Normalization of the indicators of laboratory and instrumental studies at the end of the test.
2 points	reasonable efficiency	Moderate disappearance of complaints: moderate pain reduction and secretions (points 3-4), a moderate improvement in the indicators of laboratory and instrumental studies at the end of the test.
1 point	low efficiency	The disappearance of minor complaints: a small decrease in pain and secretions (points 5-6), a slight improvement of indicators of laboratory and instrumental studies at the end of the test.
0 point	lack of efficiency	No change or worsening of clinical and laboratory parameters at the end of treatment.

Methods and timing of assessing, recording and statistical processing of performance indicators:

Registration of the performance indicators is carried out immediately after inspection and / or receipt of laboratory data. The information expressed in a quantitative form is subjected to statistical processing, including the use of special software.

The application of methods of variation statistics with the derivation of the basic parameters by Student is assumed. If necessary, multivariate analysis can be applied [4].

Criteria for assessing the tolerability of the study drug

Tolerability will be assessed on the basis of subjective symptoms and sensations reported by the patient, and objective data obtained by the researcher in the treatment process. The dynamics of laboratory indicators and the incidence and nature of adverse reactions is taken into account.

Tolerability will be assessed by the researcher, as well as patients in points:

4 points	During objective examination and/or laboratory research in the dynamics any pathological changes or clinically significant deviations are not revealed and/or a patient does not note adverse reactions
3 points	During objective examination and/or laboratory research in the dynamics minor changes are revealed that are transient in nature and do not require change of treatment with investigational drugs and/or a patient notes symptoms of minor adverse reactions, which are not causing serious problems.
2 points	During objective examination and/or laboratory, research in the dynamics significant changes are revealed that do not require additional measures and/or a patient notes an adverse reaction, which has a negative impact on his condition, but does not require discontinuation of the drug.
1 point	During objective examination and/or laboratory research in the dynamics substantial changes are revealed, and/or the patient notes an adverse reaction which has negative effects on his condition and require discontinuation of the drug
0 point	During objective examination and/or laboratory research in the dynamics significant changes are revealed and/or the patient notes an adverse reaction requiring discontinuation of the drug and additional medical measures

RESULTS

Efficacy: analysis of efficacy is performed on the results of the study on patients receiving the drug according to the scheme provided in this Protocol.

The results are provided in table 3.

Table 3. Efficiency of "MEKRITEN" developed by the Tashkent pharmaceutical Institute, Uzbekistan

Indicator	MEKRITEN		Levomycetin	
	Before	After	Before	After
Pain	0,033	0	0	0
Secretions	2,27	0	2,35	0,9

Tolerability: while analyzing side effects, it is necessary to exclude side effects that might occur from taking other medications or treatment procedures prescribed to the patient along with the test drug. If there is uncertainty, then this case can only be partly analyzed on intolerance.

Average rating of the tolerability and effectiveness in the points for "MEKRITEN" made portability of 4.97 points, and the effectiveness of 4.77 points, to 0.25% Levomycetin solution 4.8 and 3.35 points, respectively.

Both drugs did not adversely effect on the general analysis of blood.

Table 4. Changes of indicators of General blood analysis with “MEKRITEN" and 0.25% solution of Levomycetin

№	Indicators	MEKRITEN		Levomycetin	
		Before	After	Before	After
1	Hemoglobin	113,4 г/л	115,4 г/л	114,4 г/л	114,4 г/л
2	Erythrocyte	$4,14*10^{12}$/л	$4,19*10^{12}$/л	$4*10^{12}$/л	$3,9*10^{12}$/л
3	Leukocyte	$8,93*10^{9}$/л	$5,73*10^{9}$/л	$8,9*10^{9}$/л	$7,7*10^{9}$/л
4	ESR	7,07 мм/с	5,13 мм/с	7 мм/с	6 мм/с

Monitoring, audit and inspection

In the study, monitoring and auditing is made by the Customer; during the study and at the end of it inspection from the Pharmacological Committee of the MOH and GDQCM (General Directorate for Quality Control of Medicines) of the Republic of Uzbekistan is possible.

The form of release – in bottles.

Information about “MEKRITEN”

Preparation of Garlic Extract contains the oil extract of garlic as the active substances. Release form – in bottles. Storage conditions – In a dry, cool place. Shelf life – 2 years. Preparation for research and the preparation of the comparison is provided by the Contractor to the Customer free of charge. Test samples should not be used otherwise than for specified clinical studies. While receiving "MEKRITEN", no side effects and allergic reactions were observed.

The termination of the research

The research for a particular patient can be stopped if patient has adverse reactions requiring discontinuation of the drug, or the ineffectiveness of the drug in this patient, or if he missed taking the drug, or refusal of the patient from further participation in the study. There was not any of the above reasons; thus, the study was not stopped in any of the patients.

Report Registration

Clinical and analytical data obtained during this study should be evaluated and presented spreadsheets, and then summarized and discussed in the report prepared in accordance with Annex 2 to the order MOH of RUz №334 dated 25 July 2001. This study was investigated and approved by clinical ethical committee of MOH, RUz and registered N.16/2 with the date of 02.05.2014.

Publication

Using the results of the study, the Contractor undertake the first publication in a specialized medical edition. Further publications in specialized medical journals of research results can only be achieved by common consent of the parties.

Record keeping

Clinical trial documents in accordance with the List of documents of clinical trials that must be stored in a clinical database (add-on 4. to the item 5.3 of the Annex №1 to the Order of MH of the Rep Uzb. № 334 dated 25 July 2001) should be retained for at least 15 years.

Statistical analysis

All the parameters measured were expressed as means ± standard deviation. The difference between mean values was analyzed by student's *t* test at the 5% significance level. P< .05 was considered to be significant, and P > .05 was non-significant.

DISCUSSION

Garlic has been known for ages to have anti-infective properties against a wide range of microorganisms [1]. The present study has further demonstrated the antimicrobial potency of "Garlic Extract liquid" against local multidrug-resistant bacteria and Candida isolates from Uzbekistan. The observed patients were comparable to those provoked by the brand-new drug "MEKRITEN" and Levomycetin, showing that the isolates exhibited susceptibility. This indicates that "Garlic Extract liquid" has a broad spectrum of antimicrobial activity and a wide therapeutic window. The isolates tested in this study are responsible for many diseases in Uzbekistan, including bacterial meningitis, maxillary sinusitis, and otolaryngological diseases by S. pneumoniae, H. influenza and S. pyogenes [2], bronchopulmonary disorders and chronic suppurative otitis media by Pseudomonas aeruginosa [3], candidiasis and vaginitis by Candida albicans [4], nosocomial infections and bacteremia due to multidrug-resistant staphylococcal infections and diarrheal diseases caused by Escherichia coli, Shigella spp. (S. dysenteriae, S. flexneri, S. boydii and S. sonnei) and Salmonella typhimurium [2, 5]. The sensitivity of these isolates to "Garlic Extract liquid" also suggests that the intrinsic biosubstances in this extract are naive to the various drug resistance factors of the isolates, which include beta-lactamase expression, increased pyrrolidonylarylamidase activity, aminoglycoside-modifying enzymes, and altered ribosomal binding [5]. Meanwhile, the antimicrobial potency of garlic has been attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis [1, 4, 5]. Several studies, results had previously demonstrated the antibacterial potency of "Garlic extract

liquid" against enteropathogens such as Vibrio parahaemolyticus, E. coli, Klebsiella pneumonia; Proteus spp. (P. mirabilis, P. vulgaris, P. penneri, and P. hauseri); and Staphylococcus aureus [6] and anticandidal effects against Candida spp. (C. albicans, C. glabrata, C. parapsilosis, C. tropicalis and C. crusei [7]. In spite of geographical variation, "Garlic Extract liquid" for our isolates are consistent with those of Sivam [8], but are relatively lower than values obtained [9]. This antimicrobial potency disparity of garlic has been attributed to the different concentrations of individually and synergistically active biosubstances in garlic preparations coupled with their interactions with sulfhydryl agents in culture media. This phenomenon has been used to explain the stronger antimicrobial effect of allicin than garlic oil disulfides [10]. Meanwhile, allicin and other diallylsulfide compounds have been found at different concentrations in "MEKRITEN" determined by age and method of extract preparation. It can be said that the concentration at which "Garlic Extract liquid" showed growth inhibition is also fungicidal to our isolates as it displayed comparable MEKRITEN and Nystatin values. The susceptibility response observed of some of the P. aeruginosa isolates aligns with the finding [10] but at variance with the work of Kivanc and Kunduhoglu [11]. The significantly increased minimum inhibitory concentration at 48 hours postinoculation may be the consequence of colony-forming units rebound and bacteriostatic effect of "Garlic Extract liquid" on these strains. The reliability of the clinical interpretation of this observation may undoubtedly require further tolerability testing of "Garlic Extract liquid" in humans as a prelude to understanding garlic-induced plasma resistance to infections. The observation that "Garlic Extract liquid" elicited its antimicrobial potency in a dose- and time-dependent manner producing distinct time–kill profiles suggests variations in the growth inhibitory responses of the tested isolates to garlic. Similar responses have been observed and surveyed in antibiotic-resistant Escherichia coli, Enterobacter cloacae, and Citrobacter Freund [7, 12]. However, the uniqueness of time–kill profiles of the gram-positives and gram-negatives may be connected with their structurally different cell wall barriers. The lipid composition of cell wall has been found to have an influence on the permeability of hydrophobic and volatile bioactive substances in garlic [2, 14]. The eukaryotic nature and ergosterol availability in Candida cell wall may also be crucial to the observed time–kill kinetics of "Garlic Extract liquid" against our isolates [6, 9, 13].

CONCLUSION

Local therapy with the drug "MEKRITEN" (oil garlic extract) in patients with chronic suppurative otitis media is more effective than local application of 0.25% Levomycetin solution. Local therapy with "MEKRITEN" in the treatment of chronic suppurative otitis media leads to faster termination of otorrhoea and is accompanied by fewer adverse drug reactions.

According to the results of clinical trials, ototoxic properties of the drug "MEKRITEN" (oil garlic extract) in patients were not identified. The drug is well tolerated. The drug "MEKRITEN" developed by the Tashkent Pharmaceutical Institute, Uzbekistan, is fully comparable to Levomycetin.

Thus, the drug "MEKRITEN" developed by the Tashkent Pharmaceutical Institute, Uzbekistan, is effective in the treatment of chronic suppurative otitis media. The drug "MEKRITEN" developed by the Tashkent Pharmaceutical Institute, Uzbekistan, is recommended for medical use in the Republic of Uzbekistan.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author AM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author NO managed the literature searches and manuscript editing. Author SO did the manuscript review. All authors read and approved the final manuscript.

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