PREPARATION OF NANOEMULSION FROM DEER ANTLERS (CERVUS ELAPHUS SİBİRİCUS) FED İN AZERBAİJAN AND ASSESSMENT OF SOME QUALİTY İNDİCATORS

Abstract. One of the important issues of pharmaceutical technology is the creation of new dosage forms based on animal raw materials that ensure the long-term effect of medicines. One such form of medicine is nanoemulsions. Nanoemulsions are widely used in pharmaceutical systems. The nanoemulsion formula offers a number of advantages, such as the delivery of drugs, biological or diagnostic tools. The most important application of nanoemulsion is to mask the unpleasant taste of oily liquids. Nanoemulsions can also protect drugs that are subject to hydrolysis and oxidation. Currently, nanoemulsions are used for the targeted delivery of various anti-cancer drugs, photosensitizers or therapeutic agents. Nanoemulsion can also provide long-term effects of drugs. In general, all compositions of nanoemulsions can be considered effective, safe and have increased bioavailability [12]. Taking into account the above-mentioned, the optimal composition of nanoemulsions was obtained on the basis of extracts obtained by three-phase extraction from deer antlers grown in Azerbaijan.

Keywords: deer antlers, three-phase extraction method, nanoemulsion, TEM analysis, FT-IR analysis, EDX analysis, thermo-, colloid stability

Nanoemulsions are nanoscale emulsions that are produced to improve the delivery of active pharmaceutical ingredients. It is a thermodynamically stable isotropic system in which two immiscible liquids are mixed to form one phase by means of an emulsifier, i.e. a surfactant and a co-surfactant. The nanoemulsion
droplet size is usually in the range of 20–200 nm. The main difference between emulsion and nanoemulsion lies in the size and shape of the particles dispersed in the continuous phase [4,15].

It is known that the main components of the nanoemulsion are oil, emulsifiers and aqueous phases. The oils may be of any type such as olive oil, linseed oil, corn oil, coconut oil, castor oil, mineral oil, etc.

A mixture of oil and water can give a crude temporary emulsion which, on standing, separates into two separate phases due to coalescence of the dispersed globules. Emulsifiers or emulsifiers can impart stability to such systems.

Emulsifiers are broadly classified as surfactants such as spans and tweens, hydrophilic colloids such as gum arabic, and fine solids such as bentonite and veegum. The emulsifier, in addition to its emulsifying properties, must be non-toxic, and its taste, odor and chemical stability must be compatible with the product. Some of the desirable properties of an emulsifier are: (1) it must reduce surface tension below 10 dynes/cm, (2) it must be rapidly adsorbed around the discontinuous phase globule to form a complete and coherent film to prevent coalescence, (3) it must promote adequate zeta-capacity and viscosity in the system to give optimum stability, and (4) it must be effective at a sufficiently low concentration. Emulsifiers form monomolecular, multimolecular films around dispersed globules.

Ultrasonic emulsification is very effective in reducing droplet size. In ultrasonic emulsification, energy is applied through sonotrodes called an ultrasonic probe. It contains a piezoelectric quartz crystal that can expand and contract in response to an alternating electrical voltage. When the sonicator tip comes into contact with liquid, it produces mechanical vibration and cavitation occurs.
Cavitation is the formation and collapse of vapor cavities in a liquid. Thus, ultrasound can be directly used to produce an emulsion; it is mainly used in laboratories where emulsion droplets as small as 0.2 micrometers can be obtained.

The preparation of the nanoemulsion requires high pressure homogenization. This method uses a high pressure homogenizer/piston homogenizer to produce a nanoemulsion with an extremely small particle size (down to 1 nm). Nanoemulsions are a colloidal system of submicron particles that act as a carrier of drug molecules. Their size varies from 10 to 1000 nm. These carriers are hard spheres and their surface is amorphous and lipophilic with a negative charge. Magnetic nanoparticles can be used to increase site specificity. As a drug delivery system, they increase the therapeutic efficacy of the drug and minimize side effects and toxic reactions. Major uses include treatment of reticuloendothelial system (RES) infection, liver enzyme replacement therapy, cancer treatment, and vaccination [5,6,7,14].

The optimal composition of the nanoemulsion was developed on the basis of an extract obtained from deer antlers by three-phase extraction, which is rich in amino acids, hormones, enzymes and vitamins.

The first mention of the healing properties of young deer horns, otherwise called antlers, is contained in ancient Chinese mythology and in the scientific works of healers of Ancient Rome. For a long time, antlers were used as a raw material for sale to China. In the 19th century, marals began to be bred in special farms. And in the 30s of the XX century, other scientists drew attention to the popularity of antlers among their eastern neighbors and began to study their properties.
A deer farm has been established in the Khizi region of Azerbaijan. The farm, which has been operating since 2013, feeds a special species of deer (Cervus Elaphus Sibiricus) imported from Altai. In Altiagaj, which is similar to the nature of Altai, deer are allocated several hectares of forest and have all the necessary conditions for feeding these charming animals. As a result of the created conditions, the period of deer adaptation to the climate has ended successfully. At present, there are 300 deer in the deer farm. The unbone horns of Azerbaijani deer, which have sufficient raw material reserves, are used for medicinal and cosmetic purposes. Deer antlers kept here are cut in May, uncured, stored in refrigerators and used as needed [16,17,18].

The composition of deer antlers is very diverse - these are amino acids, collagen, vitamins, and macro and microelements. Among the macronutrients in the composition of antlers are iron, calcium and magnesium, sodium, phosphorus and potassium. Trace elements in antlers - manganese, selenium, cobalt. For the treatment of many diseases, deer antlers have no analogues in their effectiveness, and their healing effect persists for a long time.

It has been noticed that antlers significantly improve the condition of the musculoskeletal system, treat arthrosis and disc herniation, osteochondrosis and scoliosis, and there is also a positive effect of maral antlers in atherosclerosis, age-related changes in muscles, bones, and joints. The results of clinical studies have shown that the effectiveness of the use of deer antlers in patients with osteoarthritis is more than 90%, in patients with disc herniation - 95%, in patients with scoliosis and osteochondrosis - 98%. A noticeable reduction in pain and improvement in well-being after a course of antler therapy persists for six months.
It should be noted that on the basis of biologically active substances derived from deer antlers are produced multi-component creams, balms, ointments widely used for joint and muscle pain (Cream for the body 60 plus antlers Marala update joints, "Artrosan" - native cream for joints of maral antlers, “Artropant”, Dikulya antelope balm, etc.). However, it is not possible to obtain a high therapeutic effect in these drugs because the particle size cannot pass through the cell [8, 9,10, 11].

From the above, it is clear that there is a need to create new forms of transdermal drugs, obtained with new technology from deer antlers with a unique composition.

**The purpose of the study.** It consists of obtaining and analyzing nanoemulsions with optimal composition as a drug delivery system based on an extract from deer antlers grown in Azerbaijan.

**Materials and methods.**

Extraction of non-bone deer antlers (horns) was carried out in the laboratory of pharmaceutical technology of AMU by 3-phase extraction [1,2]. Preparation of nanoemulsion from hydrophilic and hydrophobic fractions was carried out in the laboratory of Bandel's Sonopuls HD 2070 homogenizer in the laboratory of the Department of Pharmaceutical Technology, Faculty of Pharmacy, Ataturk University. The study of particle sizes in nanoemulsions was carried out at DAYTAM (Eastern Anatolian High Technological Application and Research Center) at Ataturk University in Turkey. TEM analysis was performed on a Hitachi HighTech HT7700 device.

After the deer antlers were cut, they were frozen (4ºC) and crushed into a three-phase extraction system. Organoleptic and physicochemical properties of the extract were determined. After that, nanoemulsions were prepared in 8 variants with the participation of ultrasound to determine the optimal composition. The ratio of ingredients (aqueous-ethanol, aqueous propylene glycol, chloroform fractions of deer antlers, oily fractions of deer antlers (olive oil, flaxseed oil), spans-20, 60, 80, twins-20, 60, 80, lecithin, lecithin) By changing the mixture and the exposure time (60-240 seconds), we managed to obtain an optimal nanoemulsion. Preliminary microscopic studies have shown that the size of the nanoemulsion is 20-100nm.
Thermo- and colloidal stability and pH of the developed nanoemulsion with optimal composition were evaluated.

Zeta potential of nanoemulsions [13/Erol and Hans-Hubert, 2005], FT-IR Analysis, TEM-analysis, EDX Analysis were performe [ 3].

**Morphology**

The nanoemulsion was examined morphologically with the help of transmission electron microscopy (TEM). For this purpose, nanoemulsion was analyzed by using the “Hitachi HighTech HT7700”. The image is given below (500nm, 200nm, 100nm).

**Fig. 1. TEM image of extract loaded nanoemulsion**

**FT-IR Analysis**

The analysis was performed to examine whether an interaction had occurred in the nanoemulsion due to its blank and extract loaded form. Infrared spectra were
carried out directly on the liquid sample with the FT-IR spectrophotometer (Shimadzu IR Spirit-T) in the wavelength range of 4000-400 cm\(^{-1}\). The FTIR spectrum is given below.

![FTIR spectrum](image1)

**Fig. 1. The FTIR spectrum of blank and extract loaded nanoemulsions (red: extract loaded nanoemulsion, black: blank nanoemulsion)**

**EDX Analysis**

The elemental analysis of the extract loaded nanoemulsion was performed using the energy-dispersive X-ray (EDX) spectroscopy arranged on the scanning electron microscopy (Zeiss Sigma 300). The EDX spectrum and elemental ratio table are given below.

![EDX spectrum](image2)

**Fig. 2. The EDX spectrum of extract loaded nanoemulsion**
Table 2

Elemental ratios of extract loaded nanoemulsion.

<table>
<thead>
<tr>
<th>Element</th>
<th>Weight %</th>
<th>Atomic %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>31</td>
<td>42</td>
</tr>
<tr>
<td>O</td>
<td>39.2</td>
<td>39.9</td>
</tr>
<tr>
<td>Na</td>
<td>7.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Mg</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Si</td>
<td>17.9</td>
<td>10.4</td>
</tr>
<tr>
<td>Ca</td>
<td>2.5</td>
<td>1</td>
</tr>
</tbody>
</table>

Conclusion

During the preliminary research, it was determined that the nanoemulsion prepared at room temperature with the participation of ultrasound (70 W. 140 seconds) from the extracts obtained by three-phase extraction from deer antlers grown in Azerbaijan is 20-200 nm. Observations at 4°C, 25°C, and 45°C for two months showed that the nanoemulsion remained stable. Research in this direction is ongoing.

The nanoemulsion developed on the basis of natural components can be used in the future in the treatment and prevention of diseases of dermatology and musculoskeletal system as a system of drug delivery after extensive research.

Processed nanoematerials from deer antlers are biologically compatible and well tolerated, as well as provide a uniformly prolonged release of the active substance and have stability.

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