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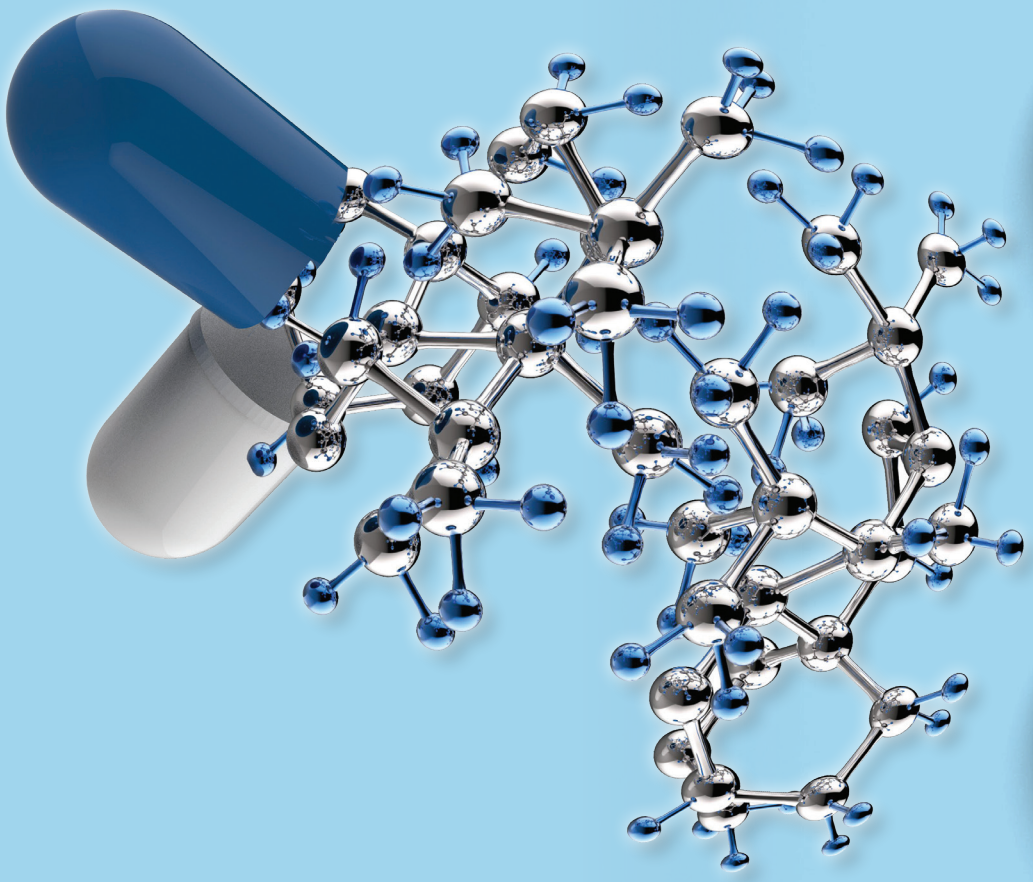

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, II, 2023 | 2-5 ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

PHARMAKEFTIKI, 35, II, 2023 | 2-5 REVIEW ARTICLE

The Adjunct Confronting Role of the Pineal Hormone Melatonin on the Bacterial and Virus Infectious Diseases

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ABSTRACT

Melatonin, a multifunctional chronobiotic neurohormone mainly synthesized and secreted by the pineal gland, plays a valuable, mainly adjunctive role in the treatment of bacterial and viral infections. In addition, the recent increase in the number of reports on the melatonin's role, as an adjuvant treatment for COVID-19, has resurged the interest of the scientific community in this hormone. With this in view, this short essay summarizes the current studies on the melatonin-promoted antibacterial and antiviral therapy, including SARS-CoV-2.

1. Introduction

Melatonin (*N*-acetyl 5-methoxytryptamine, MLT), a hormone synthesized by the pineal gland and released at night,[1] has a regulatory role in the sleep onset and maintenance in mammals, including humans[2]. It has been shown to have a hypnotic action in animals[3] and humans[4], and it has been used as an agent for restoring circadian rhythms disturbed by jet-lag, shift-work or ageing[5]. The physiological actions of melatonin, in regulating seasonal and circadian rhythms, are mediated through a family of specific, high affinity G-protein coupled membrane receptors (MT1, MT2 and Mel1c, which is not cloned from mammals)[6,7].

Melatonin has been implicated in a range of other conditions, including Parkinson's disease[8], Alzheimer's and other neurological conditions[9], and in certain cancers[10]. During the last decade various studies have illustrated the beneficial effects of melatonin on various bacterial and viral infections. Its administration has been shown to be effective in controlling chlamydial infections, infections induced by Mycobacterium tuberculosis, and also in many viral infections[11].

The present essay aims at providing the cognizant and non-cognizant reader with up-to-date information on the use of melatonin, in combating bacterial and viral infections, focusing on the multifactorial and synergic pharmacological actions of the hormone.

2. The action of melatonin against bacteriogenic infections

The protective actions of MLT against bacterial infections can occur at different levels. Direct actions of melatonin may occur only at very high concentrations, which is at the borderline of practical applicability. However, various indirect functions comprise activation of hosts' defense mechanisms or, in sepsis, attenuation of bacterially induced inflammation.

Molecular mechanisms investigations on the antimicrobial profile of melatonin suggest that this is due to its free radical scavenging potential, the direct regulation of duplication of bacteria, the depletion of intracellular substrates, like iron, etc. In view of these effects, the use of MLT, as an adjuvant or even regular therapy for serious bacteriogenic diseases, especially when no efficient direct antibacterial treatment is available, has been suggested[12-15].

3. The action of melatonin against viral infections

Melatonin cannot be classified as a viricidal drug; however, it could, indirectly, target viruses, via its anti-inflammatory, anti-oxidative and immune system enhancing actions. Melatonin has been used in the past, in experimental studies, both in *vitro* and in *vivo*, against viral infections with noticeable results [16]. In particular, it has been used against the Venezuelan equine encephalitis/encephalomyelitis (VEE) virus infection, viral hepatitis, viral myocarditis, respiratory syncytial virus infection and the Ebola virus [17].

The role of endogenous melatonin in these and other immune responses, renders the hormone a potential novel pharmacological approach in ameliorating the host reactions against viral infections and their long-term consequences. A typical example is its protecting role in infections induced by the RSV virus; in this case the hormone may at least in part prevent the injury to the airway structure through the inhibition of the oxidative stress and of the production of proinflammatory cytokine and therefore be a useful therapeutic agent in RSV-induced pulmonary disease. Recent evidence suggests that respiratory disorders induced by many other human viral pathogens may result from exuberant generation of reactive oxygen species by inflammatory cells in response to infection[18].

Melatonin has also been associated with acute liver failure, also known as viral hepatitis, which is characterized by jaundice, and the progression of encephalopathy, which leads to extensive liver necrosis[19]. A previous study suggested that the hepatoprotective role of melatonin could be noticeably induced by activating the nuclear factor erythroid 2-related factor 2 pathway, leading to the reduction of oxidative stress and the emergence of antioxidant enzymes[20]. The suppressive effects of the hormone on apoptotic damage of the liver were associated with the inhibition of endoplasmic reticulum (ER) stress, by regulating the three arms of the unfolded protein response signaling pathway[19].

Apart from the role of melatonin in treating these viruses, the hormone has been shown to have a beneficial role in treating viral myocarditis[21]. Myocarditis causes inflammation of the cardiac muscle tissues. Infectious causes of myocarditis include a wide range of parasites, such as fungi, protozoa, bacteria and viruses; however, it is most commonly associated with the inflammatory effects from viral pathogens[21]. Previous studies have suggested that apart from enteroviruses and adenoviruses, such as coxsackievirus B3 (CVB3), the human herpes virus 6 and parvovirus B19, are responsible for the emergence of cardiotropic viruses[21]. The protective role of melatonin in viral myocarditis, with a focus on the Mst1-Hippo signaling pathway, ER stress and mitochondrial dysfunction, has been investigated. It was found that the hormone improved cardiac function and repressed virus-induced cardiomyocyte apoptosis. In addition, Mst1 upregulation, caused by the virus infection, was rescued by the action of melatonin[22]. A previous study investigated the protective role of melatonin on viral myocarditis in vivo. Treatment of the hormone significantly healed myocardial

injuries, by repressing inflammation[23]. Other possible mechanisms of melatonin include the regulation of the rate of autophagy and apoptosis inhibition in mice with CVB3-induced myocarditis[23].

Furthermore, melatonin has been hypothesized to affect thrombin formation and platelet physiology, caused by the Ebola virus, which increases blood coagulation and damages blood vessels, leading to hemorrhagic shock and possibly death[17]. In addition, it has been suggested that melatonin increased the protein expression level of the enzyme heme oxygenase 1, which reduced the replication of the Ebola virus[24].

In view of the fact that melatonin has been found to have effective anti-inflammatory and anti-oxidative properties, it can be used as an adjuvant therapy in treating patients with COVID-19, with lower and predictive side effects compared to a novel drug, providing a supportive profile for a direct and safe clinical use in patients[25]. Furthermore, its safe use has been corroborated by the fact that melatonin lacks toxicity: A LD_{50} value (lethal dose for 50% of the animals used) could not be established. In addition, a high-dose of 800 mg/kg for bodyweight was not lethal[26]. Studies, which used human subjects, and were treated with varying doses of melatonin (1-6.6 g/day) for 30-45 days, have concluded that, aside from drowsiness, all other side effects (headache, stomach cramps, persistent sleepiness and somnolence) were not apparent at the end of the test period[27].

At present, there are some ongoing clinical trials, that have investigated the therapeutic dynamic of melatonin in treating COVID-19, albeit the fact that melatonin was only used in 11 clinical trials. The time spent on clinical trials, using a bioactive compound, such as melatonin, is not supported, due to the lack of protective patents for a natural compound[28]. However, this obstacle has to be surpassed, not only due to its highly safe and effective profile, but also due to its multifactorial and synergic pharmacological actions.

4. Conclusions

The recent research studies, which involve the adjunctive role of melatonin on confronting bacterial and virus infectious diseases has been highlighted collectively, in the present essay. However, more research is required, to understand the mechanism of action, at the molecular level, so that MLT's multifactorial and synergic pharmacological actions, coupled with its high safety and effective profile, could be used to treat bacterial and virus infected patients. □

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PHARMAKEFTIKI, 35, II, 2023 | 6-21 REVIEW ARTICLE

The Early Healing Arts of Ancient Greece, through the Homeric Epics

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ABSTRACT

Evidence of medical methods presence in Greece, dates back to the Bronze Age. The healing knowledge from the Homeric epics mainly concerns medicine and pharmacology, with deep knowledge of pain management, wound disinfection, and instrument sterilization. Bold operations are witnessed as early as the 6th century BC. Later, Greek and Roman doctors, for the first time, mixed medicinal plants with other common herbs without action and thus introduced the concept of excipient. Odysseus after the battle with the suitors used sulfur fumes to sterilize the Ithaca palace. For cleaning and disinfecting wounds, Limnea Earth, watered wine and copper were used, while Helen's preparation that she gave to Menelaus and Telemachus in Sparta contained opium, hemp, belladonna and mandrake. Homeric surgeons knew how to treat dislocations, remove foreign bodies from wounds, perform cauterization, apply plasters, and also relieve pain. The Hippocratics were the first to use palpation of the abdomen to find liver diseases and recognized as symptoms of liver disease as ascites, stool discoloration, fever, itching and jaundice, while as a local analgesic/antipyretic they administered Salix alba bark. At the same time, they gave great importance in body hygiene and physical exercises, both for men and women. There are indications that the caesarean section has been practiced since 500 BC. The present brief review highlights only a small sample of this precious heritage regarding the early healing arts foundations, in pharmacy and surgery, many elements of which are still applied to this day.

"This world is not ours, it is Homer's", 1180-720 BC

George Seferis, Nobel Price in literature, 1963

1. Introduction

The Greek language is one of three, which have been spoken and written for more than 3000 years (the other two being Chinese and Hebrew). At first Greek was used to keep accounting records and then to save the epics of the Iliad (almost 16000 verses) and the Odyssey (>12000 verses), which brought to life the legendary era of the Trojan War. For about a thousand years with Greek remaining the language of education in classical Greece, the Homeric epics would become a point of reference, far beyond the Greek world, e.g. Roman Virgil's Aeneid.

Until the 7th century AD, thousands of papyrus fragments of Homer's epics were found, well preserved thanks to the dry climate of the Egyptian desert. People then read and studied Homer as the closest thing to a holy book there was. For another millennium, Greek-speaking Christians would explain the ways in which the ancient epics had foreshadowed revealed Christian truths[1].

Aristotle (384-322 BC) was a philosopher with a diverse personality and multifaceted action. Apart from his philosophical works, which are completely attributed to him, as the son of Nikomachos Asklipiadis, also engaged in biological studies and experiments on animals. Nicomachos, as stated by Souidas, had written six books of medicine and one of physics, and considered his ancestor the Homeric physician Mahaon, son of Asclepius. Mahaon was mostly involved in the treatment of various wounds and diseases that required surgery, while his brother Podaleirios was the protector of "internal injuries" medicine, treating injuries of the Achaean army during the Trojan War.

According to Aristotle, medicine is divided into 5 distinct arts*: pharmacy, surgery, dietetics, nosognomy and auxiliary.* Reference by him is made to the medical treatment methods as well as attempting to highlight the surgical potential of the healers during the Homeric times[2].

Evidence of medical methods presence in Greece, dates to the Bronze Age. The warriors of antiquity from the Bronze Age to that of Rome, died of the same causes that decimated the later armies, until the "medical revolution" of the 20th century, while their weapons were just as effective in defense and attack throughout this period.

Bold therapeutical approaches have been recorded from the 2nd millennium BC. In the 6th century BC. the priests of Asclepius in Cnidus performed surgical incisions and cauterizations without anesthesia[3]. The great influence of the above accumulated knowledge based on the Greek traditional herbal medicines are found even in the byzantin manuscripts [4].

The present sort review highlights the precious heritage of the early healing arts, regarding pharmacotherapy, surgery and a number of diagnostic and treatment achievements through selective mining into Homer and classical excerpts.

1. *Pharmacotherapy attempts in classical Greece*

Greek and Roman physicians mixed the "real" medicinal plants with other common herbs that had no effect and thus introduced the concept of the excipient, an ingredient without pharmacological action, which facilitates the administration of the active drug. Examples include creating orally administered medicines with a small amount of active herb and addition of wheat or maize starch for volume increase and ease of administration. That is, they had empirically applied the concept of excipient that accompanied the dosage of the active substance. Paeon, in the E '900 of the Iliad is called to heal Mars and Hades, and to touch their wounds with powder of analgesic herbs. This powder - pulveres is still the official formulation as described in the modern pharmaceuticals[5].

In the Iliad there is the first mention of the term medicine, by Agamemnon, pa-ma-ko, who asks for Asclepius' son, Mahaon, to heal the wounded Menelaus.

Your wound will be examined by a doctor and he

will put herbs on it that will stop the black pains[3]

"Under the eyebrow he reached him, at the root of his eye, and his bulb came out he passed the eye with the pole, and on the head he projected, and he opens his arms and falls ' and Pinelaos dragging his sharp sword he lowered it, and, with his helmet on his head, threw it to the ground, and the great pole in the eye was still there like a poppy. "

It seems that the poppy was a common plant in Homeric times. Homer in the Iliad makes a simile for the warrior's eye that was thrown like the poppy leaves that float. [6]

In Homeric epics, medicine is provided without mythical or theological elements, but purely empirical, as art. It is an art practiced by skilled craftsmen, who provided their services for a fee (doctors).

Seeking healing, the ancient warrior realized that the deep knowledge of the healer was necessary, from « *ἴστημι*» *ιητήρ-γιατρός* (doctor)"i-ja-te" εγχάρακτη σε πήλινη πινακίδα γραμμικής γραφής Β. «*ιητήρ*». Besides, according to Homer:

what a doctor is definitely worth is a lot of other people: jaws erupt and emollient herbs[7] .

The "preachers", ie therapists, of the Hippocratic times were highly valued, occupying a prominent social position: The sons of Asclepius, Mahaon and Podaleirios, had important knowledge about analgesics, disinfectants and antiseptics [8,9,10].

The two brothers are for Homer the "good shepherds" who learned the precious art from their father. The services that Mahaon offers to his fellow citizens are invaluable. He knew how to pull the arrow out of their body and heal the wound with herbs, methods and ways to stop bleeding, perform surgeries, remove foreign bodies, using various bandages and applying wound patches.[11] According to Herodotus, there were medical schools even before the Hippocratic period (Kyrenia, Rhodes, Crotona, Cnidus, etc.), where the Asclepiads secretly taught medicine to their descendants, although, gradually, foreigners also learned it.

Healing was also taught by wandering therapists and medical sophists, who were not doctors but sophists and took advantage of ignorance and trust.

There were also military doctors as well as blenders, who traded medicines, poisons, cosmetics, etc., pharmacists or women pharmacists, herbalists, who sold myrrh, ointments, incense, etc., and midwifes originally from Phrygia and Thessaly, who, among other things, dealt with abortion drugs [12].

The main condition was the sterilization of the tools and the asepsis of the wound. The ancient Greeks used sulfur as a disinfectant putting importance on the sulfur content of the patches, apparently the first of the disinfectants.

Around 860 BC Asclepius discovers the plant *Vetoniki staxys officinalis*. The herb protects the liver and body from the risk of epidemic diseases [13].

Disinfection of homes, communal areas or the environment using a variety of media, such as large fires and sulfur is also reported [9].

Sulfur is reported as a mean of purification and catharsis in the hands of Achilles and Odysseus. In the Iliad, Achilles is said to use sulfur and running water to clean the well-crafted glass given to him by Thetis's mother before and then libating to Zeus[14].

In the Odyssey, after the battle with the suitors in Ithaca, Odysseus used sulfur smoke to sterilize the area.

Odysseus orders his faithful nurturer Euclid: "Bring me sulfur, which exorcises evil, old woman, bring me fire to sulfurize the palace" and he, lighting a large fire, his mansion sulfurized the beautiful" [15].

Common soil has been used as medicine since the time of Homer. Philoctetes was left in Lemnos, because the soil of Lemnos, the *Lemnos Land* as it was called, was already known for its properties, and they knew that while Philoctetes remained in Lemnos and placed the Lemnos soil often on the wound caused by the venomous snake bite, he would be cured.

Other methods of wound's disinfection was achieved using watered down wine (extremely rich in antioxidant polyphenols, acting as a bacteriostatic and bactericidal agent), vinegar, hot oil or sea water.

The disinfecting effect of *copper* was also known in Homeric times. Achilles wounded Telephus, king of Mysia, when the Greeks parked in Mysia on their way to Troy. The wound was infected with bad smell and sepsis. These wounds would be healed only, ac-

cording to an oracle, by the one who caused them "the one who creates and is healed", because otherwise the way of the Achaeans to Troy would be impossible. According to Euripides, Telephos went to Avlida disguised as a beggar and asked Achilles to cure him. Achilles refused, claiming that he had no medical knowledge but eventually, by placing fragments from his spear, the wound was healed [16].

Thus, Telephos held Orestes hostage in exchange for Achilles' help in healing his wounds. Odysseus concluded that the spear created the wound, so the spear should heal them. Today, copper has been shown to have a rapid, broad-spectrum antimicrobial activity against bacteria, fungi and viruses. It has been shown that copper shavings, when in contact with a continuous tissue solution, generate static electricity, changing the static electricity of the bacterial membrane, reducing their ability to multiply [17,18,19,20].

Humans always seek a solution to various health problems for healing and rehabilitation. The first automatic act of defense-healing in an injury or burn throughout the animal kingdom is licking, ie smearing the wound with saliva, a result of empirical knowledge, which was then shown by the beneficial presence of saliva proteins (histatins) that act decisively in closure and wound healing.

Homer introduces the concept of medicine, that is, the means of healing. When Menelaus was injured, Mahaon, after pulling the arrow from his wound, sucked it and put on a medicine (ie pharynx) that Heiron Centaurus had given to his father Asclepius During Menelaus' injury, Mahaon, the doctor, arrives and treats the wound with various herbs [21]

*"And when they (Mahaon) reached the place where the captured blonde Menelaus was standing in the middle and the strongest kings were surrounding him, the "*ισόθεος*" (isotheo) hero immediately ran and stood between them, and from the tight belt he hurriedly pulled the shawl pulling her, they broke her fingernails; And the wound, the bitter one that opened his sagitta, as he saw, the blood boiled first, and as he was holding it, after the herbs he drank, which Chiron had given to his master out of love*" [22].

The action of opium (Picture 1) on the central nervous system seems to be known to Homer. In the Odyssey Eleni is said to offer herbal wine to Menelaus and Telemachus, to deal with their grief over the absence of Odysseus. Of the many opium alkaloids, morphine is the most potent, a natural analgesic with euphoric, hypnotic and analgesic effects. Wine alcohol dissolves opium alkaloids releasing into circulation. The knowledge of the Greeks about the action of opium is obvious as shown by the representation of the goddess of the Meccans from Crete, where the codes are worn on the head. Homeric epics mention many medicines, such as "bitter" (astringent), "painkillers" (painkillers), "mild" (anti-inflammatory), "esthla" (palliative), "mitioenta" (versatile), "lygra" (palliative), "Thymophora" (deadly), "achola" (against sorrow), "charopia" like the famous nipenthes (possibly wine with opium). According to A. de Pasquale, the preparation of Eleni that he gave to Menelaus and Telemachus in Sparta contained opium, hemp, atropine (belladona) and mandrake.[23]

As anesthetics, they administered wine, opium and mandrake. In addition, high quality sanitary facilities that were discovered in the Minoan palaces prove the development of medical science in ancient Greece [24].

Various representations of the poppy in ancient art indicate that its spread in the ancient world was important, as well as the use of its juice, opium, for therapeutic purposes.

In addition to the analgesic effect (morphine, codeine) of opium alkaloids, the presence of papaverine acts as a muscle relaxant (Figure 1). They also knew from experience that alkaloids are insoluble in water and soluble in organic solvents (wine).

In Madrid there is a Greek statue of the young god Sleep, in which the god seems to be daydreaming and holding poppy seeds in his hand. Threfore, the connection of sleep with the plant is obvious and is even confirmed by Homer. The poppy causes sleep, anesthesia, muscle relaxation and memory loss [25,26].

Immediately after the initial treatment / disinfection of the wound, they would try to stop the bleeding, by using tree leaves, powders or other preparations with astringent properties completing with

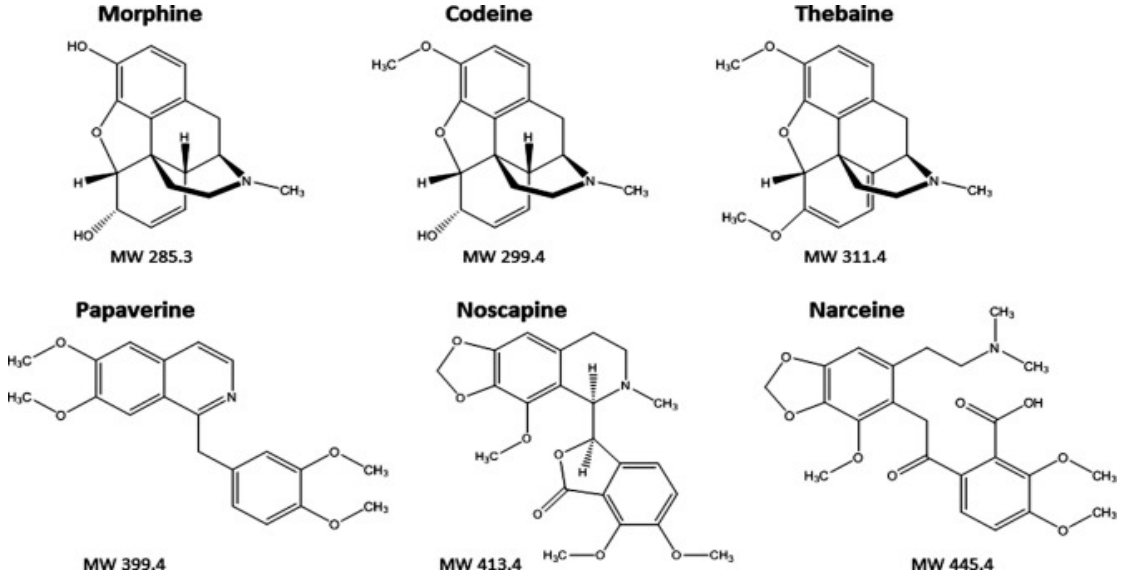


Figure 1: *Opium alkaloids*
https://ars.els-cdn.com/content/image/1-s2.0-S0308814617313808-gr1.jpg

applying the bandage on the wound[27].

Apart from the doctors, the Homeric heroes had knowledge of *traumatology* and wound care. Mahaon was taken by the tri-peak arrow that Paris fired at him, and when he came to the camp wounded, he was greeted and cared for, by old Nestor with extra care. Signs of Linear B list various plants such as fennel (*Foeniculum vulgare* Mill), dittany (*Origanum dictamnus* L.), cumin (*Cuminum cyminum* L) and celery (*Apium graveolens* L) [26,27,29].

The first known antiseptic, which is recorded on a tile of Linear B of Crete, is dittany. It was made and exported to Egypt as an antiseptic and hemostatic at the same time. They stone-crushed the leaves of the plant and placed them on the wounds. In ancient times the statue of the goddess Artemis, who was the protector of successful women, wore a wreath of dittany on her head. Also, dittany was considered capable of rejecting iron bows from wounded bodies. They used honey as an antiseptic and powerful antibiotic. Much later Galen uses the mold on wounds, which proves the early knowledge of the action of penicillin[30].

Prehistoric scholars place Asclepius in the time of the Trojan War (year of birth: 1247 BC). He produced several medicines from medicinal plants and herbs and created the famous "*drug mills*", where the processing of herbs took place. Asklipios was a resident of Trikki, and according to archaeologist P. Kastriotis, drug mills were found in Paliokastro. Asklepios collected herbs from Mount Kerketion (Koziaka), and possibly ancient Pialeia was the place where the great physician of antiquity worked and may be his birthplace. This view is further reinforced by the fact that in Paliokastro the symbols of Asclepius were found, which are the snake, the dog, the stick and the cup. Asklipios – Pialeia [31].

Apart from opium and mandrake, the ancient Greeks also used the analgesic effect of refrigerants. Cooling compounds are substances that in contact with the skin or mucous membranes give a feeling of coolness or cold. The cooling compounds exert a normal action with a chemical stimulus at the nerve endings, which creates the feeling of cold, without, however, lowering the temperature locally. Hippocrates used Mint or *Mentha Viridis* L (Pincture 2) as a local analgesic [32].

The most well-known and commonly used sub-

Picture 1: *Papaver somniferum https://upload.wikimedia.org/wikipedia/commons/thumb/4/47/Papaver_somniferum_-_K%C3%B6hler%E2%80%93s_Medizinal-Pflanzen-102.jpg/330px-Papaver_somniferum_-_K%C3%B6hler%E2%80%93s_Medizinal-Pflanzen-102.jpg*

Picture 2: *Mentha Viridis L https://upload.wikimedia.org/wikipedia/commons/thumb/0/05/Minze.jpg/330px-Minze.jpg*

stance that creates the feeling of cold is menthol, a terpenoid, the main component of the essential oil obtained from the mint plant (*Mentha viridis* L.). Other monoterpenes, present in many plants are recognized, nowadays as having high antimicrobial, antifungal, antiviral, antimalarial, antioxidant, immunomodulatory and repellent effect among other activities [33]. Furthermore, main constituents, menthol activates cryosensitive TRPM8 receptors on the skin and mucous membranes. Mint through TRPM8 receptors reduces the sensation of pain. TRPM8 is a cation channel receptor known as the TRPM8 or menthol cold receptor (CMR1). It is a protein encoded by the TRPM8 gene. With the application of cold there is a consecutive reduction: of cellular metabolism, vasoconstriction of the supply vessels initially, of blood flow, of the speed of nerve conduction, in the transport of leukocytes and mainly phagocytes, of venous and lymphatic drainage and of muscle response. Eventually, tissue metabolism is reduced locally with the application of menthol and the pain pathway is inhibited [34,35]. The Assyrians brought their male children under anesthesia through strangulation in order to perform circumcision. Another method was to hit a wooden helmet worn by the patient, in order to cause a form of concussion, carotid blockage - temporary loss of consciousness. The doctor could then perform the surgery on his patient. Anesthetic practices, perhaps not in their present form, have been practiced since ancient times by various human groups. They are as old as the Minoan civilization, as can be deduced from the study of the history of the goddess of Mycenae. The goddess of Mycenae and Yamata is a figurine that archaeologists place chronologically around 1450 BC [36].

In addition to opium, in ancient times, the "*Mandragora Officianarum* L" (Picture 3) (family of Solanides / Strychnoids) was widely used for its hypnotic properties. The active ingredients of the plant are hyoscine, atropine and scopolamine, substances that cause relaxation of LMI and inhibition of secretions. In addition, other alkaloids of the plant have analgesic properties. The use of Mandragora was known to the ancient peoples of Mesopotamia. (Akkadians, Sumerians, Babylonians, etc). The ancient Egyptians also used Mandragora as a medicine. It was discovered in the tombs of the kings of Egypt (1,800 BC). In the Old Testament and especially in the Pentateuch, the oldest nucleus of the Holy Bible (approximately 13th century BC), the aphrodisiac and fertilizing properties of Mandragora are also mentioned [37]:

Picture 3*: Mandragora Officianarum*
https://upload.wikimedia.org/wikipedia/commons/thumb/e/e4/Mandragora_officinarum_002.JPG/330px-Mandragora_officinarum_002.JPG

Picture 4*: .Ferule communis*
https://en.wikipedia.org/wiki/File:Riesenfenchel.JPG

The timely burning of corpses ensured the elemental defense against the spread of another plague or an epidemic: αἰεὶ δὲ πυραὶ νεκύων καίοντο θαμειαί. [the flames were burning and burning incessantly for the dead.]. To the heroes of Mount Athos, nothing was as dear as life and health, so illness was considered a great calamity. To prevent it, they made sure to keep their body strong. The simple diet and physical exercises helped them a lot in this, along with cleanliness (personal hygiene). The Homeric man, therefore, in order to survive during the frequent struggles he faced, had acquired basic knowledge of dealing with the wound, and was himself, the bearer of all medical knowledge. The experiences from the continuous searches, gave many opportunities to acquire such knowledge.

The nature of the battle in antiquity and the characteristics of the weapons that existed then, suggest that the chances of injury to a soldier were much higher than the chances of death. Of the 147 injuries mentioned in the Iliad, 114 (77.7%) proved fatal.

According to the Homeric epics in ancient Greece, a notable empirical medical and therapeutic method had been developed to a great extent. Doctors of the Homer era (Mahaon, Podaleirios) knew ways to stop bleeding, perform surgeries, remove foreign bodies, bandage various patches, analgesics, and apply them to a wide range of people [38-41].

2. Early surgery/orthopedic treatments

In the treatment of Orthopedic diseases, Hippocrates introduced the use of the famous "Hippocratic Ladder" as well as the Hippocratic Bank [41,42].

The fractures and dislocations were repaired using similar methods as currently applied with the use of the basic principles, traction, leverage, pressure and finally immobilization. Immobilization was achieved by using special dressings dipped in resin and wax ointment to harden. In Essence, a kind of splint was applied[43]. Hippocrates describes how to deal with almost all kinds of fractures of the human skeleton. He knows the importance of immobilizing the elbow in a flexed position for forearm fractures and the importance of the tibia versus the fork as supporting bones. In addition, Hippocrates, in selective wounds, considers "that who move after the outside" to be dangerous, extends them, straightens them and after repositioning them, then places a bandage of compressed cotton soaked in black wine and places the end in a splint from the plant Ferula the common *Ferula communis* L. (Picture 4)

The common Ferula (*Ferula communis* L) was used to stabilize limb fractures as a protector due to its flexibility, soft interior and durability.

When rearranging was very difficult, Hippocrates recommended the use of iron tools similar to levers

used in quarries by stone workers. It was applied to the length of the member as a lever as it was applied to move stone or wood. On the 7th day if the wound did not display signs of inflammation, the attempt to reposition was allowed. Removal of a bone fragment with a saw is recommended when the bone protrudes and injures the soft tissues, then a gauze impregnated with wine is placed.

Hippocrates applied an external *osteosynthesis* device to tibial fractures. Two rings made of soft leather (balls) are placed one at the height of the hammers and the other below the knee. Attached to each ring were two small leather "acorns", one inside and one outside, placed diagonally from top to bottom. The therapist had 3 pairs of rods made of flexible skull wood, about one finger thick. Two bent rods are placed in each pair of anti-diameter acorns. As the rods tend to stretch, extension is applied to the fracture site. Properly covered, it will withstand a great deal of adverse conditions and will be easily controlled [43].

He also describes the acromioclavicular dimension as a benign condition that creates only deformity and rearranges the dislocations of the temporomandibular joint.

As for the hip dislocation it describes both the anterior and the posterior, paradoxically it considers the anterior more common. Perhaps this increased frequency was the result of injuries in wrestling.

Additionally, he describes methods of repositioning both the knee dislocation and the patella. It also refers to methods to repair phalangophalangeal dislocations with the use of a special lever aid.

He describes treatment of post-traumatic edema, especially in foot injuries. In fractures of the heel, he knows the possibility of necrosis of the skin, but also as a consequence of lying down, from a bad position of the patient in bed. In his work "On Articles" Hippocrates refers extensively to *scoliosis* as well as to traumatic and tuberculous *spondylitis*. There is also a description of the clinical picture, cold abscesses and the formation of ulcers[44].

The terms *kyphosis* and *lordosis* appear for the first time in the same text [43,46].

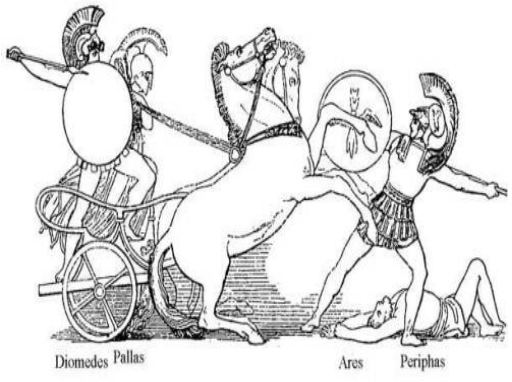
Hippocrates shows particular interest in congenital anomalies and congenital dislocation of the hip as well as talipes, which he treats as quickly as possible with manipulations and immobilization with special bandages or shoes.

The use of stretchers for transporting wounded, the so-called "mares", was also known. Injured soldiers was also transported using two or three shields, which, having been properly assembled, formed a stretcher [46]. It is also mentioned, perhaps for the first time in the history of healing, that the wounded men were transported from the field of Psachi to tents on wooden stilts called "κλισίες". The term "κλισίη" comes from the word bed (Κλίνη), which means a tent or hut made of wooden stakes, like a house made. From the description it can be derived that it referred to a kind of first aid station [47].

A study of the skeletons of Egyptian soldiers killed during a siege in 2,000 BC confirms the high mortality rate due to cranial injuries. Fifty-nine of the skeletons suffered head injuries, of which 49 were found to have been caused by stones thrown from the walls and the remaining 10 by arrows that hit the victim in the face. A 13.6 Kg stone thrown from a height of 12 meters has a kinetic energy of 1,588 Joules, capable of crushing any type of Iron Age shield[48].

Homeric heroes during the war, among other injuries, suffered fractures. The bones do not normally break due to compression (they can withstand compressive stresses of 170 MPa (megapascal) (megapascal = 2417.9683668284 kilograms-force per square). The fractures were usually due to twisting or tensile forces where the equivalent strength of the bone is reduced (120MPa). A broken bone can penetrate the skin and these fractures are more easily infected than plain ones, to which the bone is not exposed. It can be proven that that the shorter the duration of the collision, the greater the force that develops in a body as evidenced by Newton's 2nd law. That is, a reduction in the probability of a fracture is achieved when the momentum of the hit is exerted over a relatively long period of time and not instantaneously [34,49].

However, it has been observed that bones have the ability to withstand great forces even if they exceed their mechanical strength limit, provided that



Picture 5:*. The fight of Diomedes*

they are exercised for a short period of time. On the contrary, the same forces, in size and direction, are capable of causing a fracture if exercised for longer periods of time. This property of the bones is called glial elasticity.

Injuries of high kinetic energy with change of severity ≥ 2 kgr · m / sec per cm2 cause more serious injuries [50].

Medicine, as reflected in the years of Homer, had to deal with very serious bone injuries during battles. It seems that the healers of the time had a lot of experience and assisted the warriors effectively, if the wound was not fatal. In the descriptions of the Homer there are in addition about 150 anatomic terms, reference is made to epidemic diseases, plagues, snake bites, fainting spells, fevers, mental illnesses such as (mania or depression) and demonic influences[51]. Almost all types of *injuries* (140 in total) due to the weapons of the time are described, such as injuries to the head, neck, limbs, chest, internal organs - which are in fact characterized as particularly dangerous - fractures of the tibia, tibia, bones of the head [48].

It should be noted that most of these medical arts were continued by Hippocrates and others at later times [51]. Many of the anatomical terms are still used today. There are also 147 types of injuries, of which 31 are head, 16 cervical, 79 thoracic and abdominal injuries, 10 upper extremities and 11 lower extremity fractures [50]. The greatest part of the medical arts of wound healing was preserved in Hippocrates times and later.

The Iliad descriptions of the Achaean-Greek wars refer to the aggressive erosions of superficial anatomical elements and often imply damage to key organs that will lead the warrior to death without further analysis of necropsy material. Usually there is no god present[52].

Excerpts from the Iliad of the fatal injuries of the Homeric heroes are mentioned below.

1. In the file[53] mention the wounds and the fall of Deucalion by Achilles.

2. In the entry [54] is made to the clash between Diomedes and the god Aris, and Injury to Aris.

The thunderbolt was poured on Diomedes second, and his bronze pole is pushed by Athena Pallada at the bottom of Mars, where the animal is not surrounded. There he found him and tore his beautiful flesh, and the pole behind him rose up; and the iron Mars roared,

brave and shouted nine thousand, ten, mortals, tranos who started war and clashed.

The Trojans and the Argites were terrified, their offspring untied; so wild roared the unstoppable for battles Mars then,

And he quickly reached Olympus, in the immortal captivity and bitterly sat next to Zeus, the son of Saturn. He shows him the wound, the immortal that was bleeding.

3. In the entry[55] describes the fight of Diomidis Pandarou. Pandaros Death Involvement and injury of Aeneas (Picture 5).

4. Idomeneas was one of the nine candidate gladiators with Hector, aiming to end the war. Diifovos and Aeneas were his main Trojan opponents. He would have beaten Hector in the battle around Patroclus's body, but he overtook him and killed Mirionis Koiranos, the driver, so Idomeneas fled. He killed other brave warriors of the Trojans, such as Phaiston from Tarnin, son of Voros Mayonidos, Othryoneas, suitor of Cassandra.

In the entry [56], Idoeneas killed Phaistos, the son of Mayon Voros who had just arrived from Tarni. With his spear, the war-hungry Idoeneas struck him on the right shoulder, on his right as he jumped, and fell down, and a horrible darkness poured down on his eyes.

In the burial fights in honor of Achilles, Idomeneas in the post-Iliad epics, won a boxing fight, entered the Trojan Horse along with other brave Achaeans. However, being aware of the risks related to his old age, he asked for help from his comrades Achaean warriors, to face Aeneas, "the tireless murderer who has the courage and power of his youth".

"I am alone, give me your hands comrades, what are you afraid of, in front of Aeneas the agile, attacking me. With his bravery, groups of soldiers in the battle he can kill, at its peak is his youth and his strength. If only I had his youth, the young heart, who knows who would win, him or me, the victory! " He said these words, and all of them, as soon as they heard him, with a heart in their breasts, stood beside him, leaning on their shoulders [56].

5. And Idomeneus struck Eryas with a merciless rod in the mouth, and the bronze tip came out facing him, passing to the root of the brain, and smashed his white bones; from his open mouth and from his nostrils the blood flowed, and the dark cloud surround him from Charos. [57].

6. The death of Patroclus by Hector marked the course of the war [58].

Patroclus was badly hurt then and by God the blow and from the pole, he stepped back, of Charos to escape. And as Hector saw Patroclus brave then to backtrack, injured from the bronze spear, he approached him, and hit him, pushing his copper deep into his bowels. Thundering he falls, unspeakable misery for the Argites.

Homeric physicians first removed the tip of the spear or arrow, and then cleaned the wound by sucking the infected blood and rinsing it with plenty of lukewarm water. In addition, they knew how to deal with dislocations, remove foreign bodies from wounds, perform cauterizations, apply patches, but also alleviate pain with mandrakes and poppies. Finally, the wounds are bandaged with a type of gauze called a slingshot made of well-twisted sheep wool, as Homer mentions. In addition, surgical instruments have been functional since the time of Homer and were sterilized with fire or boiling oil. The slingshot bandage is later mentioned by Hippocrates and Galen [59-62].

Primitive medicine coexists with man. There is evidence that caesarean section has been practiced since 500 BC by the Greeks and the Arabs.

The caesarean section in childbirth is accompanied by a wealth of mythological elements and is associated with the birth of gods and heroes. However, it was a very old method and was applied to complicated childbirths. It was considered an emergency measure for the survival of the baby by a dying or already dead woman. In ancient mythology and legend, caesarean section gave the child supernatural powers and made him stand out above the common mortals. There are many theories as to the origin of the word *"caesarean section"* of pregnancy with the aim of rescuing the fetus. Later with the arrival of the Roman emperors the law was renamed lex caesarea, which means "imperial law". But the term caesarean section can also be attributed to Julius Caesar who was born in this way (1st century BC) The word "caesarean" was first used in the Middle Ages, derived from the Latin word caedere, a verb meaning "cut" from which the word caesura meaning a "cut" is derived [63].

According to mythology, Asclepius was the son of Koronida and Apollo. Asklepios is extracted from the womb of his mother, Koronidos, by his father, Apollo. Apollo had killed Koronida due to infidelity but saved the child. Apollo entrusted his upbringing to the Centaur Chiron who taught him to heal every disease and every wound, either with spells, or with emollient herbs, or with surgery. His daughters had names that symbolized health or medicine (*Igia, Iasso, Akeso, Panakeia*). Zeus removed Dionysus from the dead body of his mother Semeli (who had been killed by Hera in a fit of jealousy) and transplanted him to his thigh. Similarly, Adonis was removed from the body of the tree-transformed mother Myrrh [64,65].

As for the birth, Asklepios was the one who invented the "*eutokia bath*" in sea water during *dystocia*, ie the birth in water. Asklepios was deified due to his healing abilities, since the people of his time believed that he had some divine power. Thus, his fame crossed the borders of Trikki (Trikala), where he was born, covered the borders of Greece and later of the entire then known world. This event led to the

Picture 6*: Side photo with visible points of the exterior Minoan skull from the Archanes of Crete.*

Picture 7: *In the parietal bone there are (healed) signs of exostosis, which are due to ancient surgery (Photo from the Museum of the Cave of Petralona, Halkidiki).*

creation of over 340 Asclepieion during the 7th - 5th c. e.g. all over the world [66].

Apart from the myths, the archeological research proves the high level of medical knowledge of the ancient Greeks. A skull with healed *exostosis* wounds was found in Archanes, Crete, a fact that confirms that the patient survived the operation (Picture 6).

The oldest *cranial surgery* aged 28 centuries was performed in ancient Abdera of Thrace around the 7th century BC. A 20-year-old patient was wounded by an enemy missile and lived for at least 2 decades after the operation. The porous texture of the bones and the formation of new bone testify to the survival of the victim[67].

According to research by Anagnostis Agelarakis, a professor of Natural Anthropology and Archaeological Anthropology at Adlephi University in New York, the technique was similar to that described by Hippocrates in his book On Head Loss, two centuries later. In Chios, one of the ancient box-shaped tombs (second half of the 2nd century BC) contained the bones of a man, about 50 years old, whose skull had been drilled. Macroscopic examination of the hole (in the left parietal bone), 1.62 cm in diameter, showed that it was a successful surgery since the deceased lived for a long time after it. According to anthropologist Asterios Aidonis, there is a two-centimeter hole in the left back of the skull, which seems to have healed over time. The patient seems to have lived 5-6 years after the operation, while the signs of infection are minimal. The earliest evidence for the development of medical science in Greece comes from the region of Mycenae, where during the excavations a drilled skull, gallstones and skeletons damaged by arthritis were found. Everything dates back to around 1650 BC. [67-69].

3. Diagnostic-therapeutic approaches

Hippocrates lived in Greece about 300 years after the Homeric epics during the golden age of Pericles. A century of intense mental reflection that brought forth great men, such as Sophocles, Socrates and Thucydides. In the 5th c. rationalism and humanism were born, on the condition that we define this term on the broad meaning of "man's thought of himself." According to Hippocrates, the cause of the disease lies in the patient himself and the doctor needs to examine him thoroughly to reveal it. Hippocrates established the clinical examination of the patient (review, percussion, palpation, hearing - with the ear then) and proceeded to use innovative methods of treatment with herbs, decoctions, etc. Hippocrates used the willow bark for its properties. In traditional herbal medicine, Willow was widely used in fevers. It was one of the first herbs to be scientifically investigated. In the 19th century, the French chemist Leroux isolated an active ingredient called *salicin*.

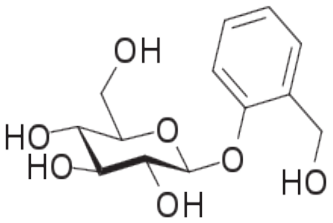


Figure 2: *Salicin (Salix alba)*
https://upload.wikimedia.org/wikipedia/commons/thumb/0/04/Salicin.svg/330px-Salicin.svg.png

(Figure 2) In 1852, this substance was produced synthetically and in 1899 acetylsalicylic acid, (*Aspirin),* was put into production. This was also the first drug of the modern generation from medicines of plant origin.

In addition, Hippocrates invented special surgical tools and proceeded to operate difficult surgeries. Such were the opening of the chest (*thoracotomy*), the drilling of the skull (for cerebral edema or tumor) etc. The surgical tools he used were scalpels, forceps, endoscopes (!), Cranial tools for skull surgeries, fetal embryos for childbirth and much more, which he used to disinfect on fire or in old wine (rich in alcohol) before using them [70].

The importance of *liver function* has been highlighted since antiquity. Homer in the Iliad states that the liver is the center of life and to give more emphasis to this view he cites the example of Achilles who was excellent and most effective in wielding the sword. With unique skill and with one blow, as he describes (Homer,) in the center of life, the liver, he thundered his opponent. It is worth noting Homer's point: with an incredible rotation of the right shoulder the sword sank and uprooted the liver. Immediately after, the white of "Mandias" was filled with brown blood that at the end was filtered with yellow drops (bile) [70].

Scientific studies on the regenerative capacity of the liver refer to Titan Prometheus, who, according to legend, was chained to a rock in the Caucasus, where every day an eagle devoured its liver, which was reborn every night.. Some believe that the myth shows that the ancient Greeks knew about the remarkable ability of the liver to self-repair. It is amazing the regenerative capacity of the liver, which is completed in the 4th week after *hepatectomy*. The myth of Prometheus mentioned by both Hesiod (8th century BC) and Aeschylus (525-456 BC) is the first record of the regenerative capacity of the liver. The *hepatoscopy* ritual seems to have increased the knowledge of the ancient Greeks about the anatomy and physiology of the liver.

Dioscorides (40-90 AD) recommends the great centaur, or silivon as a healer. Recommended for all forms of depression. In "On the Subject of Medicine" 13th c. (Oxford) Dioscorides mentions the great centaurium (*Centauria Centaurium*) as a "healing agent" and depicts the Centaur Chiron holding the plant in his hand with which he healed the wound caused by Hercules. Its healing effect of *Centauria Centaurium* was known in antiquity for liver diseases, bile diseases, snake and insect bites, poisonous mushroom poisoning, etc.

Knowledge of the healing of liver injuries also has a mythological, scientific basis. After all, according to the theory of the Indo-European language, the word "lîp" had a double meaning, denoting both life and the liver.

This divinatory art of *ionoscopy* often focused on the study of the liver more than the rest of the viscera, and fortune tellers had to have a very good knowledge of liver anatomy. It is believed that the Sumerians and the Babylonian priests first used clay models to teach liver anatomy, which also served to present the results of hepatoscopy[71].

The priests of Paphos exhibited great interest for the divinatory art of *hepatoscopy* or viscera. According to the isolated testimony of Takitos, the art of the omen scholars of the Cilician priests, the Tamirads, was foreign to the island, since the Cypriots' preference leaned towards the male carcasses and they showed greater confidence in the viscera of the goats. The inhabitants of Mesopotamia, out of an intense and inward fear for the future, were the first to systematically engage in omen studying, in particular with the older and traditional method of divination, hepatoscopy, ie examination of the liver or intestines of animals they sacrificed. A large collection on hepa-

toscopy has survived, among other divination collections.

In classical times, the knowledge about the function of the liver goes beyond the framework of beliefs and myths and is organized in a scientific way. Especially in the texts of Hippocrates and his students, which are summarized under the title Hippocratic Collection, we find elements of semiotics that are still used today in the diagnosis of liver diseases. The Hippocrates used palpation of the abdomen to diagnose liver disease and identified *ascites, stool discoloration, fever, pruritus,* and *jaundice* as symptoms of liver disease. Especially for the latter they believed that it is a result of *dyscrasia***,** attributed to an increase of bile within the body [72].

The study of the liver progressed with the work of Aristotle (384-322 BC), who dealt with both the anatomy and the physiology of the liver. Aristotle described the hepatic artery and hepatic veins and distinguished the portal vein from the rest of the hepatic venous system, while recognizing the primary role of the liver in digestion[73].

During the 2nd century BC the school of the Alexandrians, with the main representatives Herophilus (330 / 320-260 / 250 BC) and Erasistratus (c. 304-250 BC), the first physiologist and pathologist, enhanced available knowledge by a more detailed research of liver anatomy. The ability of both to perform dissections of human corpses helped them to describe the portal venous system and the bile duct. Erasistratus studied the pathophysiology of liver disease, and considered that obstructive jaundice is due to the interruption of the normal flow of bile to the bile ducts that causes inflammation of the liver and that ascites is due to sclerosis of the liver, proposing the name "schirrus", resulting in compression of the intrahepatic vessels and outflow of aqueous liquid into the peritoneal cavity. Much later Galen recognized that most of the blood is in the aorta and that blood is channeled into the aorta from the left ventricle, having previously passed through the right supplying blood to the liver. Galen also spoke about *muscle movement, respiration, semen, lung cancer, tumors, liver cirrhosis, convulsions* and *epilepsy* (the sacred disease of the ancient Greeks), *chills, tremor delirium, tuberculosis, gallstones* and *spleen diseases* [73]. But also outside of pure Greek space, Alcmaeon Crotoniatis (late 6th - early 5th century BC) a student of Pythagoras founded medicine in Lower Italy. He was the first to claim that the brain is the center of the senses and organic functions. wrote the first medical book: "On Nature" (common titles for the ancient philosophers), among others, he described the optic nerve and the Eustachian tube in the ear. He also invented surgical instruments and performed surgeries on the brain. Chalkidios describes Alcmaeon as the father of anatomy, while others describe him as the father of medicine, Alcmaeon founded medicine, and later Hippocrates transform it to science [74].

4. Conclusion

Early observations and empirical knowledge of the positive effects of the medicinal plants was the basis for experimentally determining the actual therapeutic dose that was obviously safe and no toxic. The wide range of todays well known phytotherapy and mineral effects towards infections, inflammation, wounds and pain treatments is quite impressive.

Homeric epics focus primarily in the effects of the medicines used rather than their composition, having mentioned physical exercise, diet and disorders such as epidemics, plagues, snake bites, fainting spells, fevers, mental illnesses.

In the Mycenaean Age: the disease was considered to be punishment, sent by the gods. For this reason, the treatment of patients was expected to be delivered either through direct divine intervention or through the use of knowledge related to sophisticated diagnosis in liver and nervous diseases, as well as the bold applications of thoracotomy, obstretrics, cranial surgery and bone damages.

Homer's epics are an inexhaustible and unique source for diagnosis and the healing arts in general of the time, but also it is noteworthy the great impact on the later times of Asklepios, Hippocrates Archimedes, Galen, Dioscorides and Alexandrians during prehistoric, classical and hellenistic periods. This great impact that was well preserved and spread, thanks to Greek language, is still timeless in modern science. □

Οι πρώιμες Θεραπευτικές Τέχνες της Αρχαίας Ελλάδας, μέσα από τα Ομηρικά Επη

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ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ:
ιλιάδα, φυτοθεραπεία, διάγνωση, παθοφυσιολογία, χειρουργική

ΠΕΡΙΛΗΨΗ

Τα πρώτα δεδομένα εφαρμογής ιατρικών μεθόδων στην Ελλάδα, χρονολογούνται από την Εποχή του Χαλκού. Οι θεραπευτικές γνώσεις από τα ομηρικά έπη αφορούν κυρίως τη ιατρική και τη φαρμακολογία, με βαθιές γνώσεις αντιμετώπισης του πόνου, της απολύμανσης των τραυμάτων και της αποστείρωσης εργαλείων. Τολμηρές επεμβάσεις μαρτυρούνται ήδη από τον 6ο αιώνα π.χ. Αργότερα, Έλληνες και Ρωμαίοι γιατροί ανέμειξαν, για πρώτη φορά φαρμακευτικά φυτά με άλλα κοινά χόρτα χωρίς δράση και έτσι εισήγαγαν, την έννοια του έκδοχου. Ο Οδυσσέας μετά τη μάχη του με τους μνηστήρες χρησιμοποίησε καπνισμούς θείου για αποστείρωση του ανάκτορου, της Ιθάκης. Για καθαρισμό και απολύμανση πληγών χρησιμοποιήθηκαν η «Λιμναία Γη», το νερωμένο κρασί και ο χαλκός, ενώ το παρασκεύασμα της Ελένης που έδωσε στον Μενέλαο και τον Τηλέμαχο, περιείχε όπιο, κάνναβη, μπελαντόνα και μανδραγόρα. Οι ομηρικοί χειρουργοί γνώριζαν να αντιμετωπίσουν τα εξαρθρήματα, να αφαιρέσουν ξένα σώματα και βέλη από τραύματα, να διενεργήσουν καυτηριάσεις, να εφαρμόσουν έμπλαστρα, αλλά και να απαλύνουν τον πόνο. Οι Ιπποκρατικοί χρησιμοποίησαν πρώτοι την ψηλάφηση της κοιλιάς για την ανεύρεση ηπατοπαθειών και αναγνώριζαν ως συμπτώματα ηπατικής νόσου τον ασκίτη, τον αποχρωματισμό των κοπράνων, τον πυρετό, τον κνησμό, τον ίκτερο και το φλοιό Salix alba, ως τοπικό αναλγητικό και αντιπυρετικό. Παράλληλα, έδιναν μεγάλη σημασία στην υγιεινή του σώματος και τις σωματικές ασκήσεις, τόσο για τους άνδρες, όσο και για τις γυναίκες. Υπάρχουν σαφείς ενδείξεις ότι η καισαρική τομή εφαρμόζεται από το 500 π.Χ. Η παρούσα σύντομη ανασκόπηση υπογραμμίζει μόνο ένα μικρό δείγμα αυτής της πολύτιμης κληρονομιάς σχετικά με τα θεμέλια των πρώιμων θεραπευτικών τεχνών, στη φαρμακευτική και τη χειρουργική, που βρίσκουν εφαρμογή μέχρι σήμερα.

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PHARMAKEFTIKI, 35, II, 2023 | 22-31 RESEARCH ARTICLE

Euphorbia Milii and Propolis (EMP) Combination tea Maintains Cellular Immunity in Volunteers during the Pandemic of Covid-19 without interfering with the Functions of Liver and Kidney

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ABSTRACT

Introduction: Euphorbia milii and Propolis (EMP) combination tea have proved to be immunomodulator in animal experimental study. This study aims to show the effect of the EMP tea on immune protection in volunteers during the pandemic of Covid-19 without interfering in the functions of liver and kidney. Methods: The study was performed with 30 volunteers as the subjects. The EMP tea in dose of 0.05 g per kg of body weight was prepared in tea bag and given to the volunteers. The tea was given to the volunteers at tea time once daily for 30 days during the pandemic of Covid-19 in Ketewel regency. The functions of liver (SGOT and SGPT) and kidney (BUN and SC) and cellular immune (WBC, Neutrophils, Lymphocytes and NLR) were tested before and after EMP tea intervention at day 0 and 31.

Results: The level of WBC, Neutrophils, Lymphocytes, SGOT, SGPT, BUN, and SC after EMP tea intervention were normal and there were no significant differences between before and after intervention ($p>0.05$) except for SC level, which showed significant difference before and after ($p<0.05$) although it was still kept in normal range.

Conclusion: The EMP tea protects cellular immunity in volunteers during the pandemic of Covid-19 without interfering with the function of the liver and kidney.

1. Introduction

Natural ingredients as traditional medicines are considerately easy to obtain, cheap, and have minimal or no side effects. Indonesia has a variety of natural ingredients that are trusted and many have proven their potential as drugs. The use of natural ingredients as herbal, standardized drugs, and phytopharmaceuticals must be carried out with preclinical and clinical testing stages[1.] Crown of thorns or *Euphorbia milii* (*E. milii*) flowers containing triterpenoids, saponins, phenolics, flavonoids, and alkaloids have been shown to be immunostimulants in NK cells through increased expression of NKp46[2] and expression of Interleukin-17 in mice infected with *Mycobacterium tuberculosis* (*M.tb*)[3]. Propolis has been shown as an immunomodulator against macrophage cells in TB patients[4]. *Euphorbia milii* and propolis tea is a combination of *E. milii* flower and propolis (from honeycomb). It has an immunomodulatory effect by increasing the secretion of granzyme B and did not cause lung and liver damage in mice infected with *M. tb*[5]. Giving EMP tea did not cause kidney toxicity in mice infected with *M. tb*[6]. The EMP tea at a dose of 40 mg per 100 g body weight taken once time a day (daily) has been shown to prevent atherosclerosis by lowering MMP-8 and total cholesterol but not VEGF- ß in rat with high fat diet[7]. The pandemic of Covid-19 caused an increase in morbidity and mortality worldwide. There were 448,000 cases and 14,800 deaths due to Covid-19 in Indonesia in the year of 2019[8]. The disease caused by the SAR-CoV-2 virus, like other viral diseases, is affected by the host's immune. The infection of SAR-CoV-2 virus caused an excessive host's immune response such as a cytokine storm that causes multi-organ damage[9]. For this reason, it is necessary to maintain the host's immune optimally during pandemic period with natural immunomodulators. This study aims to prove the effect of natural ingredients from *E. milii* flowers and propolis (EMP) tea for maintain cellular immune without interfere with the functions of liver and kidney in volunteers during the pandemic of Covid-19 at Ketewel Bali.

2. Materials And Methods

2.1. *The Design, place, and time of Study*

The study was an experimental study with 30 volunteers as subjects, and they were tested for complete blood count (CBC) for data of the cellular immunity at before and after EMP tea intervention. The subjects consisted of 17 females and 13 males according to inclusion and exclusion criteria i.e physical examination, liver and kidney function within normal range. The study was done at Udayana University (the Laboratory of Food Processing Faculty of Agriculture Technology, the Laboratory of Integrated Biomedical Faculty of Medicine, the Laboratory of Analytic) and the Mantra Medica Clinical Laboratory at Ketewel, Gianyar, Bali. The study was conducted for 6 months in the year 2021.

2.2. *Materials*

Euphorbia milii (*E.milii*) flowers, honeycomb or tala, tea paper bag, reagen from Erba for SGOT (Serum Glutamic Oxaloacetic Transaminase), SGPT (Serum Glutamic Piruvic Transaminase), BUN (Blood Urea Nitrogen), SC (Serum Creatinine) and reagents from Sysmex (cell pack, stromatolites and cell clean) for CBC (Complete Blood Count).

2.3. *Instrumentations*

Blender (Philips series 5000) to cut E.milii flower and honeycomb into a small pieces; Dryer Oven (Memmert UN55) to dry E.milii flower and the honey comb; impulse heat sealer to seal tea bag; Hematology analyzer (Sysmex XP 100) to process complete blood count such as WBC, neutrophils, lymphocytes; Chemical analyzer from Erba XL 100 to process the blood chemistry test as SGOT, SGPT, BUN and SC; disposable syringe 5 ml (BD), blood collecting tube, centrifuge tube, centrifuge (WINA instruments; type 507; rotor 12 tube; Centrifuge Hi Speed Electromotor to separate serum from the blood), spectrophotometer (UV-1800; Shimadzu UV Spectrophotometer serial no A114549 O7235) to examine the phytochemical content of EMP tea.

2.4. The Preparation of EMP tea

E.milli flowers are picked from a flower plantation in Ketewel, Gianyar, Bali. Approximately 1000 grams are cleaned and put in a drying oven at a temperature of 40 0C for 24 hours. Drying flower then was blended and then 400 grams of E.*milii* powder was produced. Propolis were obtained from the honeycomb which was found on a honey bee farm at Plaga, Badung, Bali. Approximately 1200 g of honeycomb or tala which was been empty and cleaned, then was cut into small pieces before dried in a drying oven at a temperature of 40 0C for 48 hours. Approximately 600 g of honeycomb powder were produced and ready to be mixed with *E.milii* powder. The EMP tea from *E.milii* flower and propolis (tala) was made by mixturing powder of 400 g *E.milii* and 600 g tala. After the two ingredients are mixed well, it is ready to be packed into the tea bag. The doses 0.05 g per kg of body weight daily were prepared in tea bag and then brewed with 100 ml hot water and ready to consume after cooling.

2.4. *Phytochemical and nutritional examination of EMP tea brew*

The EMP tea brew was examined for its phytochemical and nutritional in accordance with standard procedure content by laboratory staff of the Analytical laboratory at Udayana University.

2.4.1. Total Phenolic

Determination of total phenolic by Folin–Ciocalteau method [10.] Folin-Ciocalteu reagent was diluted with water at a ratio of 1:9 (v/v). To in 1.25 ml of reagent, 50 µl of sample was added. After that, it was incubated for 2 minutes at room temperature, then added 1 ml of sodium carbonate (75 g/l). The mixture was incubated for 15 minutes at 50°C and quickly cooled in container filled with ice water. In 15 minutes the absorbance was read at wavelength 760 nm. Reading results were compared with the standard curve which was constructed using gallic acid at concentrations of 0, 25, 50, 75, 100, 150, 200 ppm. The standard curve equation is:
y=0.0023x+0.0057, R^2=0.9991.

2.4.2. Total Flavonoid

A total of 1 ml of sample was mixed with 4 ml distilled water and 0.3 ml of NaNO2 solution (5%). After 5 minutes, 0.3 ml of AlCl3 solution (10%) was added, then vortexed and left for 6 minutes. Then 2 ml of NaOH solution (1 M) was added and 2.4 ml of distilled water. The absorbance of the solution was measured at a wavelength of 510 nm. The quercetin standard curve was prepared using concentrations 0, 2, 4, 6, 8, 10, and 12 mg/ml. The standard curve equation is: y=0.0011x+0.0017, R^2=0.9997.

Concentration of flavonoids in the test sample is calculated from the calibration standard curve and stated as quercetin equivalents in mg/g sample[11]

2.4.3. IC_{50} (Antioxidant activity)

A total of 3 ml of DPPH (0.004% w/v in methanol) was dissolved in 100 µl of avocado leaf extract (1% concentration) in a test tube. The solution was incubated for 30 minutes in the dark at room temperature. The absorbance is read at a wavelength of 517 nm against control using methanol as blank. The percentage of the ability to capture free radicals (antioxidant activity) is calculated by equation (1):

$$\% = \frac{Ao - As}{Ao}\ x100$$

Where Ao : the control absorbance and As the sample absorbance.

Next the calculation results are entered into the regression equation Y = aX + b.

Where Y: % inhibition value (activity antioxidant) and X: the extract concentration (100-2000 mg/L). $IC_{50\ v}$alue is derived from the calculation 50% inhibition[12]

2.4.4. Vitamin C

Vitamin C or ascorbic acid (AA) determination

Table 1: The general characteristics of volunteers

Characteristics	Volunteers
Age (years old)	**Persons (%)**
25-35	11 (36.67)
36-46	19 (63.33)
Sex	**Persons (%)**
Male	13 (43%)
Female	17 (57%)
Physical Examination (general sign)	**30 persons (100%)**
Blood pressure (mmHg)	110/70 – 125/85
Resting heart rate (beats per minute)	70 – 85
Respiratory rate (breaths per minute)	16 – 20
Body Mass Indexes /BMI (%)	19.5 – 22

was performed by simple redox titration methods using 2.6-Dichlorophenolindophenol (DCPIP). A solution of DCPIP was calibrated by 2 mL standard solution of AA, which contained 1.08 mg. Based on the obtained data each mL of DCPIP solution was equivalent to 0.093 mg of AA. Then the samples of EMP brew were titrated by calibrated titrant. After titrations, results were expressed as mean ± confidence limits at 95% confidence level[13]

2.4.5. β-Carotene

The concentration of β-carotene was determined using U-HPLC. The U-HPLC system (LaChromUltra L-2000 U Series; Hitachi-High Technologies Corp., Hitachinaka, Japan) was equipped with an eluant reservoir, a U-HPLC pump (Model L-2200U), an autoinjection system of 5 µL injection at a fixed volume. LaChromUltra C18 (2 µm, 2 mm i.d.×50 mm L, Hitachi-High Technologies Corp.) was used as an analytical column. Mobile phase was ethylacetate: acetonitrile: acetic acid (30:68:2, v/v/v) with 0.22 mM BHT and flow rate was 0.2 mL/min. Detector was UV detector (Model L-2400U; Hitachi-High Technologies Corp.) set at the wavelength of 450 nm. Calibration graph for U-HPLC was based on peak area and prepared by injecting 5 µL of 0.5, 1.0, 5.0, and 25.0 µg/mL solutions prepared by the dilution of β-carotene stock solutions with a mobile phase[14]. The standard curve equation is: y=174.33x-18.35, R^2=0.999

2.4.6. Mineral composition

Mineral composition of the samples was determined according to methods recommended by Association of Official Analytical Chemists [15]. The samples were incinerated in the oven at a temperature of 550°C for 3 hours. The samples of EMP tea was digested using a mixture of concentrated Nitric (HNO_3), perchloric ($HClO_4$) and sulphuric ($H2SO_4$) acids in the ratio 9:2:1 (v/v) respectively. Copper (Cu), iron (Fe), zinc (Zn), sodium (Na), calcium (Ca) and magnesium (Mg), manganese (Mn), and lead (Pb) were determined by Atomic Absorption Spectrophotometer (AAS) (PerkinElmer Analyst 700, England). Details are explained in Adjatin *et al.* (2013)[16]

2.4.7. Proximate analysis

The sample was analysed for moisture, crude protein, and ash content. Crude protein was determined by using the Kjeldahl method. The moisture was determined according to the procedure of Association of Official Analytical Chemist (AOAC, 1990).

The percentage was calculated based on the dry weigh. Ash was determined after incineration in a muffle furnace[16]

2..4.8. Sugar

Total sugars in the samples of EMP tea brew were determined according to methods recommended by AOAC 968.28-1969 (2000)[17]. And the result of total sugars were expressed in percentages.

All experiment were carried out in triplicate and the results were represented by mean and standard deviation. The phytochemical results are presented in the Table 2.

2.5. *The intervention of EMP tea for the healthy volunteers*

The volunteers were given EMP tea in dose of 0.05 g per kg of body weight daily for 30 days. The EMP tea was given during tea time, between 10 am – 12 am. During the EMP tea consumed, the research has only standardized the diet only for the prohibition of vitamin and other nutritional supplements, whereas the daily meal was according to the subject's usual diet.

2.6. *The liver functions (SGOT and SGPT) and kidney functions (BUN and SC) tests at before and after EMP tea intervention*

The volunteers with normal clinical signs were approved and informed and provided consent. The diet control during the test is according to the protocol of variable of interest, i.e fasting for 8 hours prior testing (only drinking water were allowed). Approximately 2 ml of blood were collected in a tube without anticoagulant from mediana cubiti vein with a standard procedure. The blood sample was then processed according to the procedure of an Erba XL 100 chemistry analyzer with Erba reagen for SGOT, SGPT, BUN, and SC. The test was done before and after EMP tea intervention.

2.7. *Determination of cellular immunity from complete blood count (WBC, lymphocytes, neutrophils and NLR)*

Blood samples from volunteers were collected by laboratory staff according to standard procedures. Two ml of blood was taken from Mediana Cubiti vein and were put into an anticoagulant tube for a further process according to standard procedure for complete blood count examination using Sysmex XP100 hematology analyzer with reagent cell pack, stromatolites, and cell clean from Sysmex.

2.8. *Statistical Analysis*

The data of immune cells (WBC, lymphocytes, neutrophils and NLR), liver functions (SGOT and SGPT) and kidney functions (BUN, and SC) on before and after EMP tea intervention were tabulated, described, and analyzed using SPSS version[24].

3. **Results and Discussion**

3.1. *Results*

The general characteristic of volunteers are shown in Table 1. There were 30 healthy volunteers who qualified, consisting of 43% male and 57% female. The mean age was 36,67% between 25 - 35 years old and 63,33% between 36 – 45 years old. All volunteers (100%) have normal body mass index (BMI), blood pressure, heart rate, temperature, and respiratory rate. They did not have any complaints such as fever, coughs and colds.

The feature of cellular immune, liver and kidney function, of the volunteers is described in Figure 1. The features of immune cells in the volunteers could be seen from CBC i.e the number of white blood cells (WBCs), neutrophils, lymphocyte and NLR in before and after EMP tea intervention. The mean number of WBC before and after intervention were still in the normal range (ref. value in male: 4.1-11x 10^3/µl; female 4.1-11x 103/µl). The same condition happens in the mean of the neutrophil percentage before and after EMP tea intervention was also in the normal limit (ref. value: in male: 50-70%; female: 20-60%). Similarly with the mean of lymphocytes percentages in before and after EMP tea intervention were also in the normal limit (ref. value in male: 13-40%; in female: 30-64%). From the results, there were no

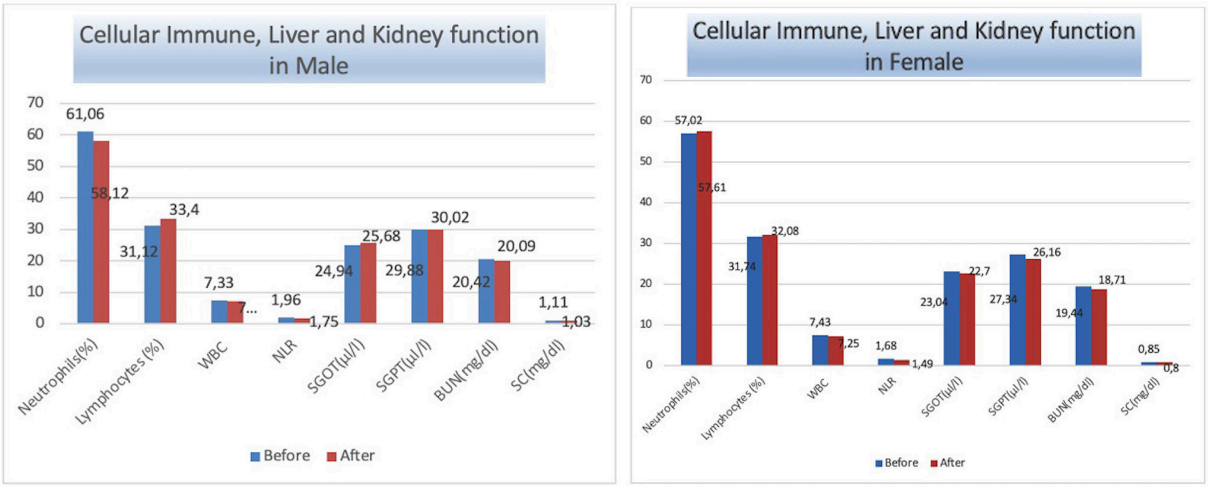

Figure 1. *The Feature of Cellular immune, Liver and Kidney functions in Volunteers*

significant difference between before and after EMP tea intervention in the number of WBC, neutrophil percentage and lymphocytes percentage of the volunteers. There were also no significant difference in the neutrophils-lymphocytes ratio (NLR) between before and after EMP tea intervention. It seems that giving EMP tea is able to replace the role of multivitamins or supplements that might be consumed before the tea intervention in maintaining cellular immunity of volunteers. In male, the mean level of SGOT and SGPT before and after EMP tea intervention was still in the normal range (reference value for SGOT: 10-40 µl/l; SGPT: 10-55 µl/l). Similarly in female, the mean level of SGOT and SGPT before and after the administration of EMP tea was also in the normal range (reference value for SGOT: 9–25 µl/l; SGPT: 7 – 30 µl/l). The result also showed no significant difference ($P > 0.05$) between before and after intervention values of SGOT and SGPT both in male ($P = 0.822$) and female ($P = 0.863$). The mean value of kidney functions (BUN and SC) in male and female in before and after EMP tea intervention were shown in normal limit. In which the normal value in male (BUN: 8-25 mg/dl; SC: 0.6-1.5 mg/dl) and female (BUN: 8-25 mg/dl; SC: 0.5-0.9 mg/dl). The result showed there are no significant difference ($P > 0.05$) between before and after EMP tea intervention in BUN level but it was significant difference in SC level ($P = 0.030$). It seems, EMP tea intervention did not interfere with liver and kidney function in volunteers so it was safe for consumption.

The results of the phytochemical and nutritional examination of EMP tea are described in Table 2. The EMP tea brew contains polyphenols, flavonoids, antioxidants, vitamin C, beta carotene, glucose, protein, Fe, Zn, Ca, Mg, Mn, Na, water content, ash content, Cu, and Pb. The EMP tea brew has good content and did not contain heavy metals in concentrations that are harmful to the volunteers.

3.2. Discussion

This study is a continuation from previous EMP experimental study in mice and rats. Previous studies concern the effect of *Euphorbia Milli* tea and its combination with propolis on a number of glomeruli in *Mycobacterium tuberculosis* infected mice. It was found that the Euphorbia milii tea and its combination with Propolis does not affect histological change on the total number of glomerulus in mice infected with *M. Tb*[7] so EMP tea does not impair renal fuction.

Other studies found that *Euphorbia milii* and propolis combination tea (EMP) did not cause liver toxic-

Table 2: The Phytochemical and nutritional content of EMP tea brew

Parameter	Unit	Results
Polyphenols	mg/mL GAE	55 ± 0.05
Flavonoids	mg/mL QE	1.32 ± 0.01
IC_{50}	mg/mL	1.65 ± 0.02
Vitamin C	mg/mL	13.2 ± 0.05
β-Carotene	mg/mL	0.979
Water content	%	99.895 ± 0.02
Ash content	%	0.003 ± 0.0001
Protein	%	0.077 ± 0.002
Sugar	%	0.08 ± 0.002
PH	A-	6.2 ± 0.01
Iron (Fe)	ppm	7.44
Zinc (Zn)	ppm	16.6
Calcium (Ca)	ppm	24.6
Magnesium (Mg)	ppm	5.49
Manganese (Mn)	ppm	0.083
Sodium (Na)	ppm	6.77
Copper (Cu)	ppm	2.419
Lead (Pb)	ppm	0.204

Resource: Testing report no: 58A/UN.14.24/UPTLA/2021

ity and lung damage while affecting granzyme secretion in *Mycobacterium tuberculosis infected mice*6. It has also been reported that EMP combination tea increases the diameter of lymphoid nodule (white pulp) in the rat spleen[18]. Moreover, *Euphorbia milii* flower and propolis (EMP) combination tea prevents atherosclerosis through decreasing MMP-8 and total cholesterol level but not VEGF-β in rat with high-fat diet (HFD)[7]. In connection with immunomodulator potency and lack of liver and renal toxicity in animal experimental studies above, the present follow up study aims to prove the protective effects of EMP tea in volunteers during the pandemic of Covid-19, without interfering with the functions of the liver and kidney. The results of the liver functions (SGOT, SGPT) and renal functions (BUN, SC) in before and after the EMP tea administration showed normal limits, although there was a significant difference ($P < 0.05$) in the level of serum creatinine (SC) between before and after EMP tea intervention both in male ($P = 0.030$) and female ($P = 0.044$). After 30-day administration of EMP tea there was a decrease in SC level. This condition indicated a a favorable condition, which is beneficial and may be partly contributed to EMP tea administration. The presence of phytochemicals and nutrients in the EMP tea brew i.e polyphenol, may have a protective effect, as supported by Fernandes and Costa.[19]. The EMP tea also contains flavonoids which have been shown to reduce kidney damage[20]. Iron (Fe) in EMP tea has also a protective effect on kidney function[21] as well as beta carotene[22]. Zinc in EMP tea also supports kidney function. Zinc deficiency has been reported to be a risk factor for chronic kidney disease progression[23]. The infection by Sar-CoV-2 caused the interaction of virulence agent with the host immunity. The main

immune response to viral infections was innate and acquired cellular immune responses. In general, cellular immune responses to viral infections virus are mediated by white blood cells (WBC) which circulate throughout the body through blood vessels and migrate to lymph nodes via high endothelial venules (HEV). The regulation of the immune system can be assisted by using immunomodulator substances from natural resources i.e EMP tea[7]. The excessive immune response to SAR-CoV-2 infection has been reported to induce a cytokine storm due to excessive pro-inflammatory cytokines that damage tissues and then cause various multi-organ failure (MOF), which ends in death9. The mean of neutrophil percentage before and after the EMP tea intervention was in normal limit. Similarly was the mean lymphocytes percentage. Neutrophil- lymphocyte ratio (NLR) values before and after intervention were also within normal limits. but there was a decrease in NLR although the decrease was not statistically significant. The decline in NLR values during the pandemic of Covid-19 indicates a positive situation, where high NLR values are often associated with an inflammatory state[25]. The flavonoid have previously been studied to have an effect on NLR values. Flavonoid in Dayak onion bulb extract increase the production of IL-2 which is involved in the activation and proliferation of lymphocytes. Flavonoids also have an anti-inflammatory potential through their ability to modulate the expression of pro-inflammatory genes[26,27]. The other compounds and minerals in EMP tea such as zinc (Zn) and iron (Fe) reduce NLR. This was supported by research from Zhou et al28 . Iron deficiency causes immune system dysfunction and affects the proliferation of T lymphocytes. Other studies reported that zinc was a micronutrient with its anti-SARS-CoV-2 viral effect and it could modulate the immune response. Deficiency of selenium, iron, and zinc is associated with the abnormal regulation of neutrophils and lymphocytes in Covid-19 patients and influences NLR and severity of the disease[29,30,31].

The present study shows, that EMP tea maintains optimal cellular immunity (WBC, neutrophils, leukocytes) as before tea intervention. The volunteers consumed immunomodulatory supplements or other multivitamins freely before tea intervention, while during EMP intervention for 30 days they were free from any supplement. This indicated that EMP tea has an immunomodulatory effect so that cellular immunity conditions remain optimal even during the Covid-19 pandemic and its also safe for the liver and kidney. □

4. Conclusion

The administration of EMP tea with a dosage of 0.05 g per kg of body weight daily for 30 days maintains cellular immunity of volunteers without interfering with the functions of the liver and kidney during the pandemic of Covid-19 in Ketewel, Bali.

Ethics Committee

This research has received ethical approval from the research ethics committee of the faculty of Medicine Udayana University according to number: 2607/UN14.2.2.VII.14/LT/2021.

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Conflict of interest

The Authors declare no conflict of interest.

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PHARMAKEFTIKI, 35, II, 2023 | 32-41 RESEARCH ARTICLE

Thermodynamic Functions of Chromatographic Retention of Sodium 2-((4-Amino-5-(thiophen-2-ylmethyl) -*4H*-1,2,4-triazol-3-yl)thio) acetate and its Impurities

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ABSTRACT

The aim of this work was to study the dependence of liquid chromatography retention on temperature and thermodynamic characteristics of the transfer of sodium 2-((4-amino-5-thiophen-2-ylmethyl)-*4H*-1,2,4-triazol-3-yl)thio) acetate, 2-(thiophen-2-)yl)acetohydrazide and 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-*3H*-1,2,4-triazole-3-thione from the mobile phase to the stationary. HPLC system Agilent 1260 Infinity. Column Zorbax SB-C18; 30 mm x 4.6 mm; 1.8 μm. The mobile phase consisted of water (0,1% HCOOH) and acetonitrile (0,1% HCOOH) (75:25). Flow rate is 0.4 mL/min. The solutions of each substance were chromatographed at different temperatures from 30°C to 70°C with step 5°C. The sodium 2-((4-amino-5-thiophen-2-ylmethyl)-*4H*-1,2,4-triazol-3-yl)thio)acetate and its technological impurities are characterized by negative values of the enthalpy of transfer from the mobile phase to the stationary, so the process of transition of the substances from the mobile phase to the stationary prevails over the reverse process. The entropic term more than enthalpic term for the 2-(thiophene-2-yl)acetohydrazide at the influence on retention.

1. Introduction

Derivatives of 1,2,4-triazole-3-thione exhibit a variety of biological activities, so and they attract a lot of attention from researchers looking for new biologically active substances[1-6].

Sodium 2-((4-amino-5-thiophen-2-ylmethyl)-*4H*-1,2,4-triazol-3-yl)thio) acetate is an active pharmaceutical ingredient (API) with potential actoprotective action[7-9].

Thermodynamic characteristics is an important factor in the control of retention in high-performance liquid chromatography (HPLC). The temperature allows controlling the selectivity of the chromatographic separation, and it is also known that with increasing temperature the viscosity of the eluent decreases, which, given the pressure limitations during the operation of the column, is useful and requires detailed study. Due to this factor, water dissolves non-polar compounds better, because the dielectric constant of the medium decreases. The thermostat of the column allows you to avoid fluctuating retention when the room temperature changes[10]. Thus, the study of the thermodynamic parameters of retention of substances, firstly, has a theoretical value, because it helps to clarify the nature of retention, and secondly, it has a direct practical value, because it helps to choose the optimal separation conditions.

Studying the thermodynamics of the retention allows us to better clarify the nature of the chromatographic mechanism as reflected in partitioning, London dispersion bonds, dipole-induced, dipole-dipole, ion-dipole, hydrogen bonds, ionic bonds, etc. Linear dependence of retention factors ln$k$ on $T^{-1,}$ as deduced from the Van't-Hoff equation, allows predicting the dependence of chromatographic behavior at different temperatures based on a small number of experiments[11].

The thermodynamic characteristics obtained by the Van't-Hoff equation also allows describing the properties of the column sorbent and comparing it with other sorbents[11, 12]. Further elucidation of the retention mechanisms will help to more efficiently select and create new stationary phases, as well as change the mobile phase to improve the selectivity of chromatographic systems for the determination of given analytes. Evaluation of thermodynamic characteristics helps to determine whether enthalpic or entropic control prevails in the adsorption of analytes. Many scientists pay attention to the study of thermodynamic parameters of retention[13–25]. Most relevant studies however over the past 20 years have been conducted mainly for enantioselective sorbents on chiral stationary phases[13-22]. Therefore, the study of classical octadecyl silica gel systems deserves more attention.

The aim of this work was to study the dependence of the retention on temperature, as well as a the thermodynamics of mobile to stationary phase transfer of sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)thio) acetate and its impurities-2-(thiophen-2-)yl)acetohydrazide and 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione - using a C-18 column.

Derivatives of 1,2,4-triazole-3-thione exhibit a variety of biological activities, so and they attract a lot of attention from researchers looking for new biologically active substances[1-6].

Sodium 2-((4-amino-5-thiophen-2-ylmethyl)-*4H*-1,2,4-triazol-3-yl)thio)acetate is an active pharmaceutical ingredient (API) with potential actoprotective action[7-9].

Study of thermodynamic parameters of sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate, 2-(thiophen-2-)yl)acetohydrazide and 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione retention was held for the first time.

2. Materials and methods

2.1. Chromatographic equipment.

High-performance liquid chromatographic system Agilent 1260 Infinity consisted of degasser (Agilent Technologies, Japan), binary pump (Agilent Technologies, Germany), autosampler (Agilent Technologies, Germany), thermostat column compartment (Agilent Technologies, Germany), diode array detector (Agilent Technologies, Germany). Software was OpenLAB CDS.

Chromatographic conditions.

Column was Zorbax SB-C18; 30 mm x 4.6 mm; 1.8 μm. The mobile phase consisted of water and acetonitrile (75:25) in presence of 0,1% HCOOH. The pH of the water:acetonitrile mixture was measured about 2.8. Flow rate was 0.4 mL/min. The wavelengths of the diode array detector were 232, 234, 246 nm. Injection volume 2 μL.

2.2. Reagents.

Sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl) thio)acetate (1), 2-(thiophene-2-) yl)acetohydrazide (2), 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3) were synthesized at the Department of Natural Sciences for Foreign Students and Toxicological Chemistry of Zaporizhzhia State Medical and Pharmaceutical University. Direct Q 3UV (Millipore, Molsheim, France) was used for the production of high purity water (18 MΩ, 25°C). Acetonitrile HPLC Super Gradient grade (Avantor Performance Materials Poland S.A., Poland), formic acid «For analysis» (98 %) (AppliChem GmbH, Germany).

2.3. Preparation of solutions. Solutions of sodium

2-((4-amino-5-thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (**1**), 2-(thiophen-2-)yl) acetohydrazide (**2**) and 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3**) were prepared at a concentration of 0,001% in the mixture of water and acetonitrile (75:25*).*

2.4. Determination of temperature dependence. The final solutions of each substance were chromatographed at different temperatures from 30^{o}*C to* 70^{o}*C with step 5* ^{o}*C*, under conditions described in 2.2.

2.5. Calculation of octanol water distribution coefficients logD.

Octanol -water distribution coefficients logD at pH 3 calculated using the on-line service[26]

2.6. Statistical Analysis

Microsoft Excel was used for regression analysis and graph construction

3. Results and discussions

Structures of the sodium 2-(thiophene-2-yl)acetohydrazide (1), 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-thione (2) and sodium 2-((4-amino-5-thiophen-2-ylmethyl)-*4H*-1,2,4-triazol-3-yl)thio)acetate are shown at Fig. 1.

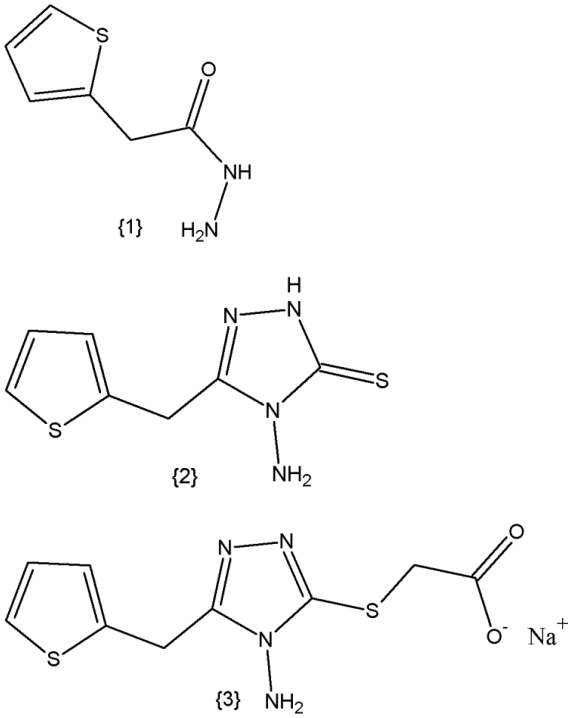

Figure 1. *Structures of studied compounds (1-3).*

The optimal parameters (maximum separation at maximum retention) are observed at 25% acetonitrile and 40°*C* of the column thermostat. Retention of each substance at optimal conditions is shown at chromatograms was acquired at the wavelengths of maximal light absorption (Fig. 2).

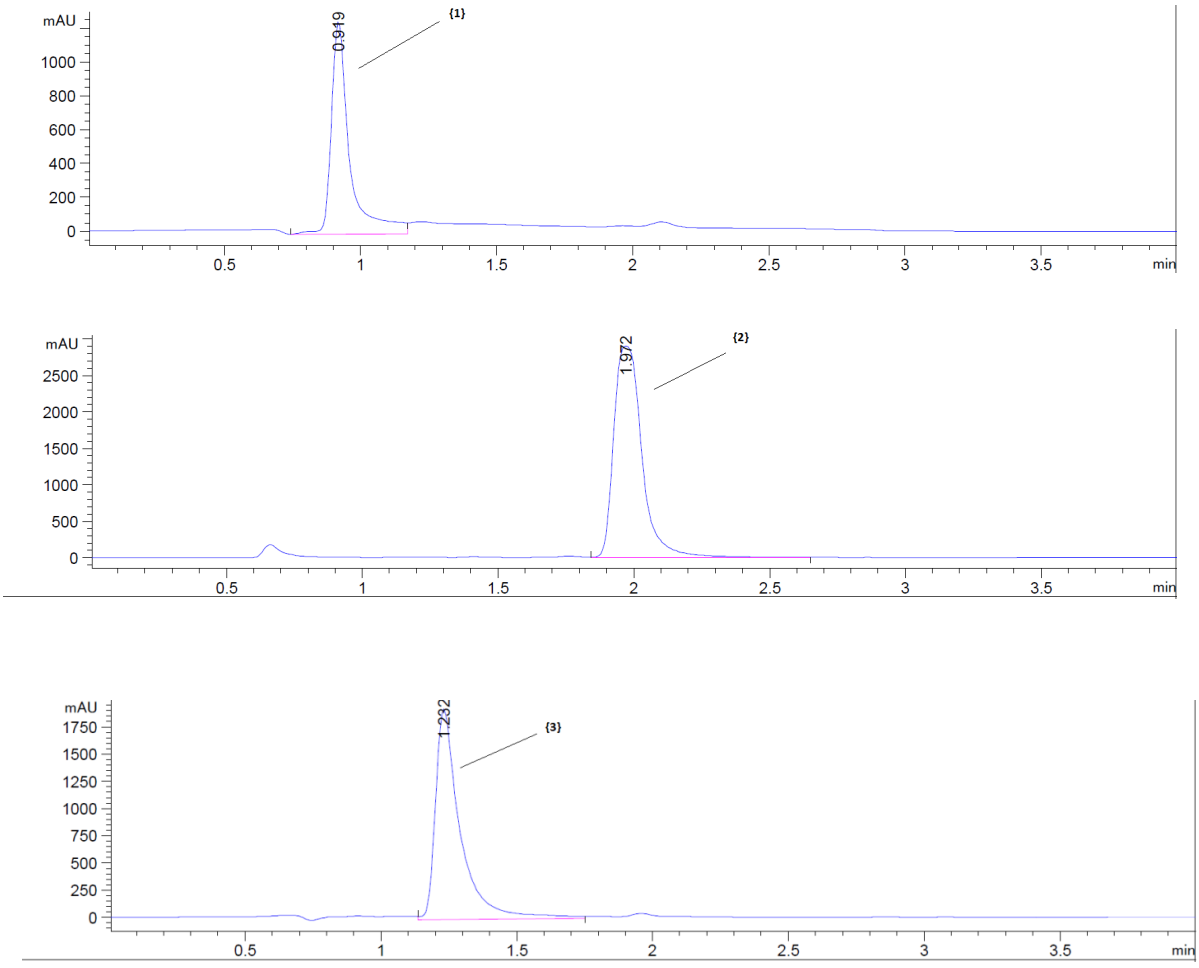

Figure 2. *Chromatograms of compounds (1) at 234 nm, (2) at 246 nm, (3) at 232 nm.*

For the study of the thermodynamic parameters of the transfer of the analyte from the mobile phase to the stationary retention factors are determined according to equation (1):

$$k = \frac{(t_r - t_0)}{t_0} = \frac{V_S}{V_M} \quad (1)$$

where t_r - retention time,

t_0 - retention time of an unretained substance determined using KNO_3 and equal to 0.6 minutes.

$\frac{v_S}{v_M}$ – the ratio of the amount of substance analyte (mole) in the stationary phase to the amount of substance analyte (mole) in the mobile phase.

The ratio of the volume of the mobile phase to the stationary β , also known as the phase ratio, or phase volume ratio is given by equation (2)[11]

$$\beta = \frac{V_M}{V_S} \quad (2)$$

The equilibrium constant *K* of the transfer of substance from the mobile phase to the stationary is described by equation (3):

$$K = \frac{[A]_S}{[A]_M} = \frac{v_S}{v_m} \times \frac{V_M}{V_S} = k \times \beta \quad (3)$$

where $[A]_S$ – concentration of analyte in the stationary phase,

$[A]_M$ - concentration of analyte in the mobile phase.

The well-known Vant-Hoff's equation (4):

$$lnk = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (4)$$

$$ln(k \times \beta) = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (5)$$

where β – equation (2)

Based on equation (5), equation (6) is formed:

$$lnk = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} - ln\beta \quad (6)$$

Equation (6) is reduced to a linear form, (equation (7)):

$$y = mx + b$$

where $y = lnk$, $x=T^{-1}$, $m = -\Delta H^0/R$, $b = \Delta S^0/R - ln\beta$.

The standard enthalpies of transfer of analytes from the mobile phase to the stationary phase may be calculated on the basis of equations (6) and (7):

$$\Delta H^0 = -m \times R \quad (8)$$

So, the dependence of the retention factor on temperature was determined for each substance, and on the basis of the obtained results, the enthalpy of transfer of the substance from the mobile phase to the stationary one was determined.

Figure 3 shows the effect of temperature on the chromatographic retention of compound (3).

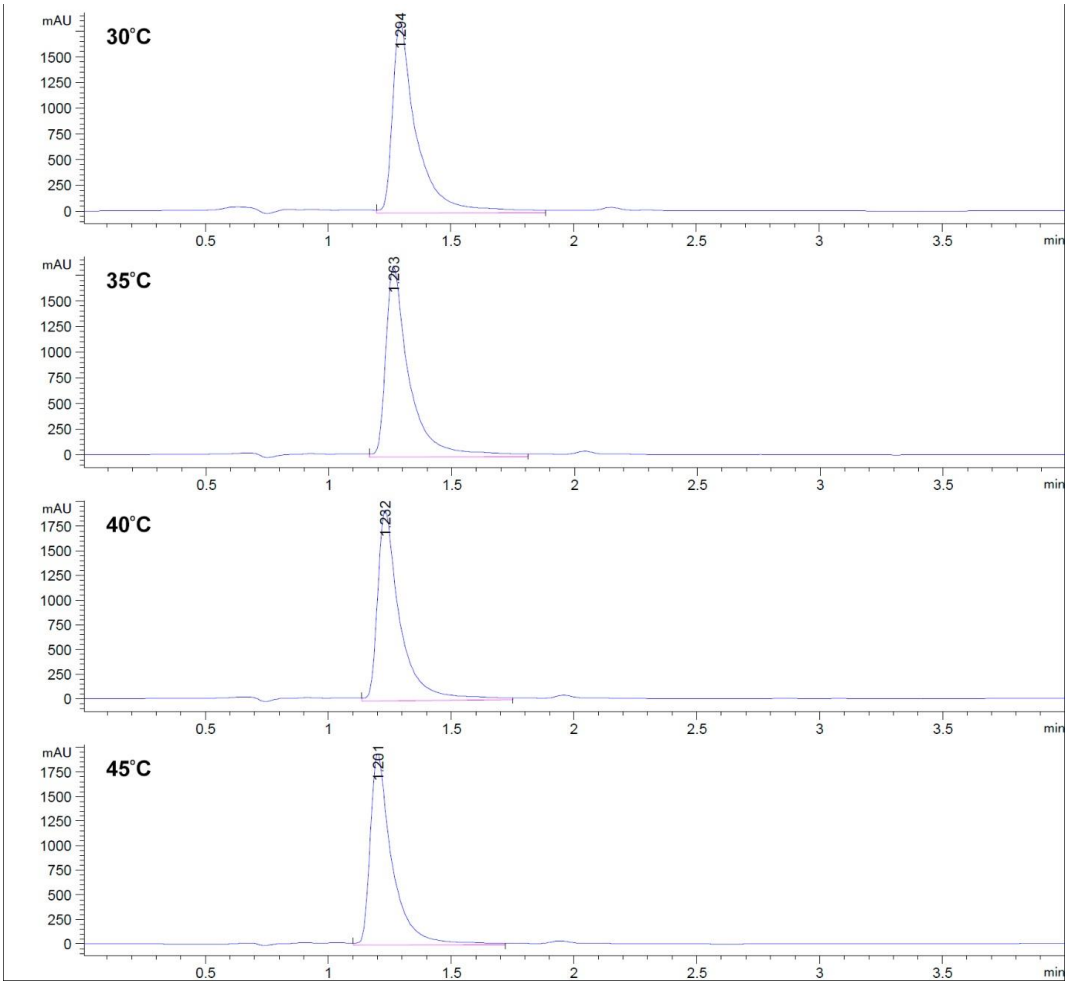

Figure 3

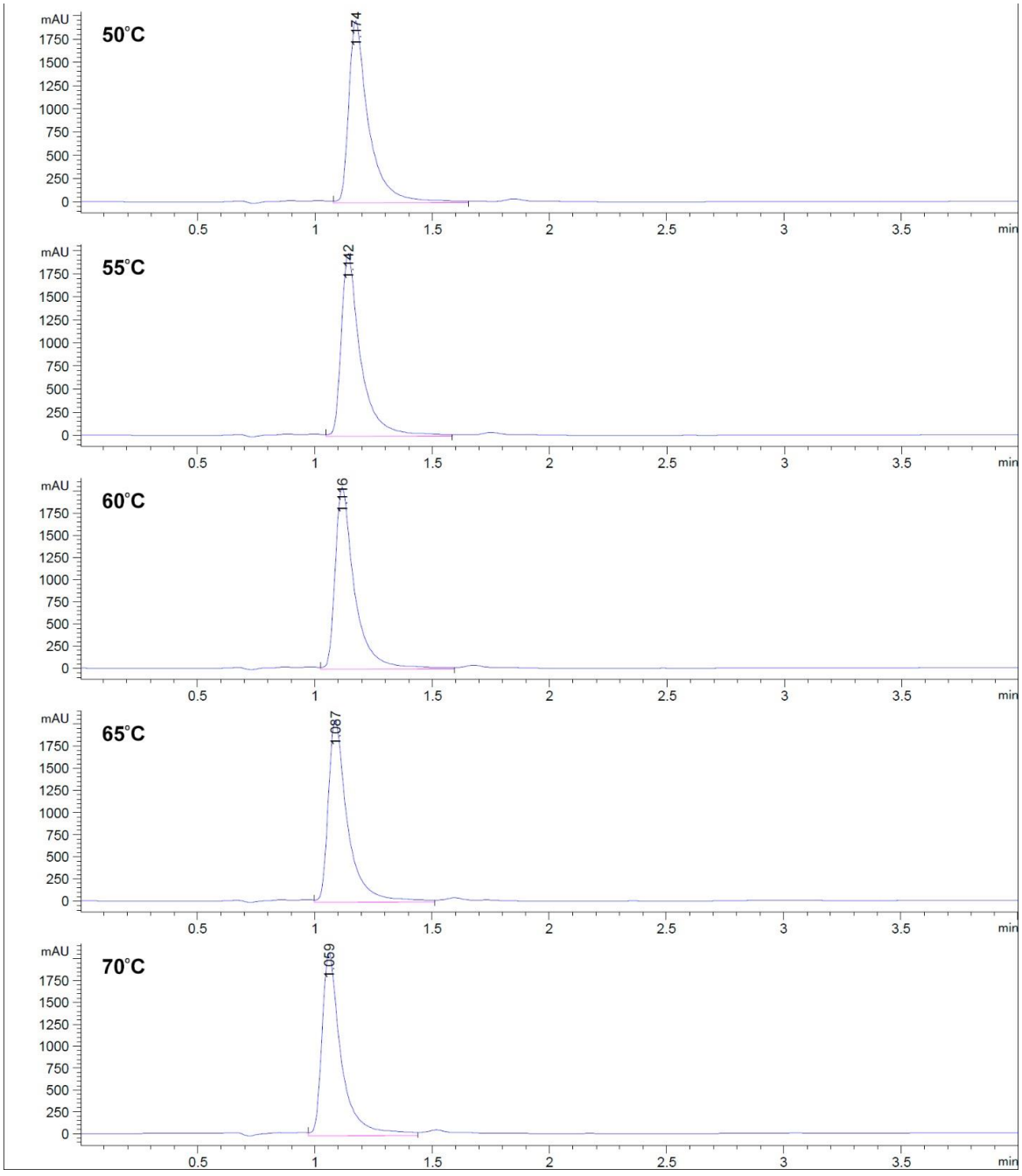

Figure 3, *continued. Chromatograms of compound (3) at different temperature values (30–70°C).*

Average retention time after six measurements in the same temperature values for substances at temperatures from 30 °C to 70 °C with step 5 *°C were used for* calculation of retention factor *k*.

Equations of linear dependence of *lnk* on T^{-1} were calculated using the program Microsoft Excel by the method of least squares (Table. 1) and the corresponding graphs were built (Fig.4).

Table 1. Equation of linear dependence lnk on T-1

Substance	Equation	R^2	*S* (standard regression error)
1	$lnk = 214.49\ T^{-1} - 1.318$	0.938	0.007767
2	$lnk = 817.35\ T^{-1} - 1.088$	0.995	0.007885
3	$lnk = 1088.8\ T^{-1} - 3.439$	0.994	0.,012108

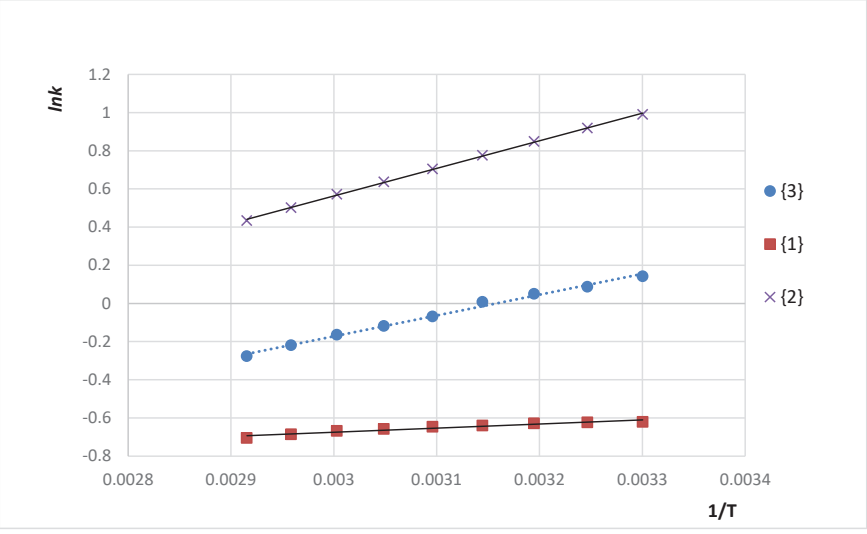

Figure 4. *Graph of dependence lnk on T^{-1} for compounds 1-3.*

The values of R^2 for the compounds (2),(3) higher than 0,99 supports the linear nature of the dependence as a result of homogenous reversed phase retention mechanism. As for substance (1), which is a highly polar molecule, the lower R^2 may indicate the contribution of secondary retention mechanisms most likely ion-exchange due to its interaction with free silanol groups.

The standard enthalpies of transfer of the analyte from the mobile phase to the stationary one were calculated using the equation (8) as follows.

Calculations for 2-(thiophene-2-yl)acetohydrazide (1):

ΔH^0=-214.5×8.31=-1780 J/mol=-1.78 kJ/mol

For 4-amino-5-(thiophen-2-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (2):

ΔH^0=-1443.2×8.31=-11990 J/mol=-11.99 kJ/mol

For sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (3):

ΔH^0=-1088.8×8.31=-9050 J/mol=-9.05 kJ/mol

Calculated enthalpies are summarized in Table 2 along with the corresponding logD values at pH 3.

Octanol-water LogD as a lipophilicity (hydrophobicity) measure influences the retention mechanism in particular in reversed-phase systems. Indeed higher ΔH^0 by absolute value corresponds to the higher *LogD* values and thereupon and to higher retention factor.

According to equation (6), retention is affected by both the enthalpic term $-\Delta H^0/RT$ and the entropic term $\frac{\Delta S^0}{R} - ln\beta$. According to the Table 2 enthalpic term increase retention because positive but enropic term decrease retention because negative. Also analysis of the Table 2 show that for compounds (2) and (3) the enthalpic term contributes more than entropic term by absolute value. So these compounds better retained due to more hydrophobic interaction with stationary

Table 2. Standard enthalpies of transfer of analytes from the mobile phase to stationary and LogD of compounds

Substance	$-\Delta H^0/RT$, J/mol	$\frac{\Delta S^0}{R} - ln\beta$, J/mol K	ΔH^0, kJ/mol	$LogD$
{1}	0.720	-1.32	-1.78	0.06
{2}	4.84	-3.77	-11.99	1.24
{3}	3.65	-3.44	-9.05	0.2

phase and have more ordered state. However for compound (1) prevails the entropic term. Indeed, the retention of compound (1) has the lowest value, denoting that the compound is distributed more in the mobile phase, which corresponds to a more disordered state.

4. Conclusion

Standard enthalpies of transfer of analytes from the mobile phase to the stationary one are determined for all substances. The sodium 2-((4-amino-5-thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)thio) acetate and its technological impurities are characterized by negative values of the enthalpy of transfer from the mobile phase to the stationary, so the process of transition of matter from the mobile phase to the stationary prevails over the reverse process. The entropic term influences more than enthalpic term the more polar compound 2-(thiophene-2-yl)acetohydrazide.

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, ΙΙ, 2023 | 42-51 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 35, II, 2023 | 42-51 RESEARCH ARTICLE

Research of Anti-inflammatory and Oncoprotective activity of the *Hosta lancifolia* dry Extract on the Model of DMH-induced Carcinogenesis

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ABSTRACT

The purpose of this work was to study anti-inflammatory and oncoprotective properties of the dry extract from hosta lancifolia leaves in an experiment on rats with a chemically induced tumor process

Materials and methods. The experimental work was performed on 120 white male rats. Chronic oncogenic intoxication was modeled by administering 1,2-dimethylhydrazine hydrochloride for 30 weeks (1 time per week). A dry extract from the leaves of hosta lancifolia was administered intragastrically daily at a dose of 100 mg/kg of the animal's body weight throughout the experiment in order to correct the detected disorders. Blood serum of rats was taken for research monthly. The level of cytokines, in particular, interleukin-4, interleukin-6 and tumor necrosis factor-α was determined by the immunoenzymatic method. The content of C-reactive protein was determined by the immunoturbidimetric method.

Results. It was found that the daily use of a dry extract from hosta lancifolia reliably reduces the level of pro-inflammatory interleukin-6, tumor necrosis factor-α and C-reactive protein, increases the level of anti-inflammatory interleukin-4 in the blood serum of rats under the conditions of carcinogen long-term use.

Conclusion. The obtained results of the study give grounds for asserting that the extract from Hosta lancifolia at a dose of 100 mg/kg exhibits a pronounced anti-inflammatory and omcoprotective effects under the conditions of induced carcinogenesis, which creates a perspective for further study of the extract as an oncoprotector.

1. Introduction

Taking into account the urgency of finding new effective drugs with low level of toxicity, allergenicity and a wide range of therapeutic action, the attention of scientists is drawn to drugs that are of natural origin. In particular, preparations from plant raw materials and mushrooms are increasingly used in the complex therapy and prevention of liver diseases, cardiovascular system disorders and oncopathologies[1, 2].

Oncological diseases are especially dangerous, because there are no symptoms in the early stages of the disease. According to the World Health Organization (WHO), cancer is the sixth leading cause of death worldwide. Ukraine is among the countries with a high incidence of cancer. Every year, about 65,000 people die from cancer, and 140,000 learn about their disease [3] . Cancer prevention is aimed, primarily, at the elimination of carcinogenesis – the process of tumor initiation and development. Primary prevention is, first of all, the elimination of carcinogenic factors, most of which are caused by lifestyle, and the maintenance of health in general. Quitting smoking, changing the diet, adding vitamins and microelements, timely treatment of chronic diseases may reduce the probability of getting cancer [4, 5] . The use of oncoprotectors is promising for the prevention and treatment of oncological diseases, especially as a part of complex therapy [6] .

In this aspect the objective of the present research was hosta lanceolate (Hosta lancifolia Engl.), which, due to its rich content of biologically active substances, can exhibit appropriate pharmacological activity [2] . In particular, hosta leaves contain polysaccharides, carboxylic acids, compounds of steroid and phenolic nature, volatile compounds, sesquiterpene lactones, saponins, flavonoids, carotenoids, which allows us to suggest its anti-inflammatory, antioxidant, membrane-stabilizing properties and the possibility of use hosta lancifolia as an oncoprotector [7, 8] .

The aim of experimental work was to study anti-inflammatory and oncoprotective properties of the dry extract from hosta lancifolia leaves using experiments in rats with chemically induced tumor process.

2. Material and methods

2.1. Material

Experiments were performed on white outbred male rats weighing 190-210 g, which were kept on the standard diet of the vivarium of I. Horbachevsky Ternopil National Medical University. All studies were conducted in compliance with Good Laboratory Practice (GLP) and bioethics in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [9] . The study was approved by the Ethical Committee of I. Horbachevsky Ternopil National Medical University (Excerpts from the protocol №70, from 01.08.2022).

2.2. Experimental induction of oncopathology

We chose 1,2-dimethylhydrazine hydrochloride (1,2-DMH) (SIGMA-AL DRICH CHE MIE, manufactured in Japan, series D161802) to simulate chronic oncogenic intoxication. This chemical compound causes carcinogenesis in the intestines of rats after long-term administration. 1,2-DMH undergoes biotransformation in the liver, forming metabolites that, entering the intestines, cause the development of tumors. This model of the oncopathology development is sufficiently studied. It has also been confirmed that neoplasms that arise in experimental animals are similar in their morphological structure to similar human neoplasms [10] .

The carcinogen was previously diluted with an isotonic sodium chloride solution before administration. The 1,2-DMH solution was injected subcutaneously into the interscapular area at a dose of 7.2 mg/kg (based on the active substance) once a week for 30 weeks, according to the rat's body weight [10] .

2.3. Experimental design

The experimental rats were divided into three groups: 1st – control (C); 2nd – animals affected by 1,2-dimethylhydrazine hydrochloride, control pathology (CP); 3rd – rats affected by 1,2-dimethylhydrazine hydrochloride, treated with a hosta lancifolia leaves dry extract (HLLDE). HLLDE was administered intragastrically daily at a dose of 100 mg/kg of animal body weight during 30 weeks of the experiment [3] . Rats, which were injected subcutaneously with saline every week, were the control for experimental group of animals.

Animals were euthanized and blood was taken from the heart of the rats once a month. Thiopental anesthesia was

used for all manipulations. To obtain blood serum, blood samples were allowed to coagulate for 30 min at room temperature, then centrifuged for 15 min at 1200.

2.4. Methods

The content of C-reactive protein (CRP) and the cytokine profile were determined. Cytokine profile was assessed by the levels of pro-inflammatory (tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6)) and anti-inflammatory IL-4 cytokines in blood serum. The concentration of interleukins in the blood serum of rats was determined by the immunoenzymatic method using commercial kits ("GE Health care: Amersham", Great Britain) [11] . The quantitative level of TNF-α in blood serum was determined by the method of competitive immunoenzyme analysis in vitro using the immunoenzymatic test system Rat Tumor necrosis factor α ELISA Kit MBS (USA) according to the manufacturer's method, which was described in technical documentation [12] . The CRP content was determined by the immunoturbidimetric method [13] using a semi-automatic biochemical analyzer Humalyzer 2000.

2.5. Histopathological studies

The collection of material for histological studies was carried out according to generally accepted methods [14] . Pieces of rat colon were fixed in a 10% formalin solution, while the duration of exposure did not exceed 1-2 days. The applied fixing solution prevents the process of autolysis and stabilizes cells and tissues for their further processing and use in staining procedures. Next, the pieces were dehydrated in alcohols of increasing concentration in an AT-4 machine for histological processing of tissues, and embedded in paraffin blocks. Sections with a thickness of 5-7 μm obtained on a sledge microtome MC-2 were stained with hematoxylin and eosin. The slides were observed under light microscope under ×100 magnification.

2.6. Statistical analysis

Statistical analysis of the data was performed using STATISTICA 13 (TIBCO Software Inc., 2018). Parametric and nonparametric methods of evaluation of the obtained data were used for statistical processing of the results. For all indices, the arithmetic mean of the sample (M) and the error of the arithmetic mean (m) were calculated. The reliability of the difference between the values between the independent quantitative values was determined by the Mann-Whitney test. The difference between the values was considered probable at $p<0.05$ [15, 16] .

3. Results

Analyzing the obtained results, it was found that the administration of 1,2-DMH to white rats caused an increase in the level of CRP, pro-inflammatory cytokines IL-6, TNF-α and a decrease in the level of anti-inflammatory cytokine IL-4 in the blood serum of animals, which confirms development of the inflammatory process.

3.1. Effect of Hosta leaves extract on the CRP level in the blood serum of rats

C-reactive protein is a criterion of inflammation severity and a strong marker of cardiovascular risk. CRP is synthesized in the liver in response to the action of proinflammatory cytokines (IL-6, IL-1, transforming growth factor β, TNF-α), participates in complement binding and macrophage phagocytosis [17, 18] . One of the leading functions of the CRP is the elimination of foreign pathogens, including endotoxins. CRP also helps in cleansing the body of necrotized or apoptotic cells. The concentration of CRP is usually proportional to the inflammation intensity level, because this marker is sensitive to the smallest changes in the acute phase response [19] . The level of CRP in blood serum rapidly decreases with the completion of inflammatory process, as it has a short half-life (4-7 hours).

An increase in the content of CRP in the blood serum of rats with a simulated tumor process was established throughout the study. Thus, the level of CRP in animals injected with 1,2-DMH increased by 1.3, 1.9, and 3.1 times compared to the control on the 3rd, 5th, and 7th month of the experiment (Table 1).

Table 1: Content of C-reactive protein in the blood serum (mg/l) of rats affected by 1-2-DMH and after the use of hosta lancifolia leaves extract (M±m; n=120)

Period of affection	Index/Group of animals		
	C	CP	CP+HLLDE
1 month	3.79±0.18	3.86±0.26	3.89±0.20
2 month	3.79±0.18	4.13±0.16	4.09±0.19
3 month	3.79±0.18	5.05±0.19*	4.65±0.19
4 month	3.79±0.18	6.46±0.24*	5.14±0.24**
5 month	3.79±0.18	7.22±0.28*	6.00±0.18**
6 month	3.79±0.18	9.41±0.23*	6.67±0.20**
7 month	3.79±0.18	11.62±0.25*	7.78±0.15**

*Note. Here and in the following tables * - probable changes between the index of control and dimethylhydrazine-affected animals, ** - probable changes between the index of dimethylhydrazine-affected and extract-treated animals.*

Table 2: Content of IL-6 in blood serum (pg/l) of rats affected by 1-2-DMH and after the use of hosta lancifolia leaves extract (M±m; n=120)

Period of affection	Index/Group of animals		
	C	CP	CP+ HLLDE
1 month	3.14±0.14	3.34±0.15	3.41±0.16
2 month	3.14±0.14	3.88±0.25*	3.64±0.16
3 month	3.14±0.14	4.75±0.22*	3.87±0.17**
4 month	3.14±0.14	6.02±0.32*	4.63±0.11**
5 month	3.14±0.14	8.07±0.47*	5.47±0.23**
6 month	3.14±0.14	9.40±0.33*	6.04±0.22**
7 month	3.14±0.14	11.40±0.54*	6.81±0.16**

In the group of animals that received dry extract from the leaves of hosta lancifolia in parallel with the toxicant, a probable increase in the level of CRP was observed from the 4th month of the study compared to the control pathology.

3.2. Effect of hosta leaves extract on the IL-6 level in the blood serum of rats

One of the proteins whose level increases during inflammation is IL-6, which is an active cytokine that takes part in the implementation of the immune response and development of the inflammatory reaction [20, 21].

Activated monocytes or macrophages, endothelial cells, fibroblasts, activated T-cells, as well as cells that are not immunocytes produce IL-6. It is the main factor in the growth and regulation of the differentiation of activated B-lymphocytes into antibody-producing cells, stimulates the synthesis of fibrinogen, CRP and other pro-inflammatory proteins [22].

Administration of 1,2-DMH to white rats for the pur-

Table 3: Content of IL-4 in blood serum (pg/l) of rats affected by 1-2-DMH and after the use of hosta lancifolia leaves extract (M±m; n=120)

Period of affection	Index/Group of animals		
	C	CP	CP+ HLLDE
1 month	1.34±0.06	1.39±0.08	1.46±0.07
2 month	1.34±0.06	1.27±0.08*	1.23±0.05
3 month	1.34±0.06	1.13±0.06*	1.20±0.07
4 month	1.34±0.06	0.99±0.06*	1.14±0.02**
5 month	1.34±0.06	0.82±0.10*	1.08±0.03**
6 month	1.34±0.06	0.68±0.06*	0.97±0.04**
7 month	1.34±0.06	0.53±0.07*	0.88±0.06**

pose of oncopathology modeling led to a probable increase in IL-6 content in the blood serum of animals by 1.5, 2.6, and 3.6 times on the 3rd, 5th, and 7th months of the study respectively, in relation to control group (Table 2).

In the group of animals to which HLLDE was administered after 3 months from the beginning of the experiment, the studied indicator decreased by 18.5% compared to the control pathology, after 5 months - by 32.2%, and after 7 months - by 40.3%, respectively.

3.3. Effect of Hosta leaves extract on the IL-4 level in the blood serum of rats

The next stage of our research was the study of IL-4 content in the serum of animals. IL-4 is an anti-inflammatory cytokine produced by mast cells and type II T-helpers, suppresses the release of pro-inflammatory cytokines IL-1, IL-2, IL-6 and IL-8 [23, 24] .

According to the obtained data, the content of IL-4 in the blood serum of rats with a simulated tumor process probably decreased starting from the 2nd month of the study (Table 3).

As it is evidenced by the data shown in Table 3, the IL-4 content in blood serum had a tendency to increase on the 3rd month of HLLDE using as a corrective agent, but it probably did not differ from the control pathology. On the 5th and 7th months of the study, in the group of animals receiving HLLDE, a probable increase in the level of IL-4 was noted by 31.7% and 66%, respectively, relative to the affected rats.

3.4. Effect of Hosta leaves extract on the TNF-α level in the blood serum of rats

One of the most important mediators of the acute phase of inflammation, in addition to IL-6, is TNF-α. Therefore, determining the content of IL-6 and TNF-α in blood serum makes it possible to assess the functional activity of various types of immunocompetent cells, the severity of the inflammatory process, and predict the course of the disease [12, 25] .

TNF-α has a multifaceted effect on the protective functions of the body, on the development and maintenance of the functional organization of lymphoid organs, necessary for an optimal immune response. It is known that a physiological function can be positive or negative. The destructive effect of TNF-α is manifested in the case of a severe inflammatory process and autoimmune diseases [12, 25] .

A significant increase in the level of TNF-α was noted throughout the experiment (Table 4). Thus, an increase in the content of this indicator in the rats' blood serum was observed by 1.8, 4.2 and 6.9 times, respectively, after 3, 5 and 7 months from the beginning of 1,2-DMH administration, in relation to the group of affected animals.

Table 4: The level of TNF-α in the blood serum (ng/l) of rats affected by 1-2-DMH and after the use of hosta lancifolia leaves extract (M±m;n=120)

Period of affection	Index/Group of animals		
	C	CP	CP+HLLDE
1 month	1.08±0.09	1.21±0.11	1.27±0.11
2 month	1.08±0.09	1.39±0.10*	1.37±0.07
3 month	1.08±0.09	1.89±0.10*	1.64±0.08**
4 month	1.08±0.09	2.91±0.18*	2.26±0.13**
5 month	1.08±0.09	4.56±0.19*	3.24±0.21**
6 month	1.08±0.09	6.11±0.29*	4.01±0.26**
7 month	1.08±0.09	7.44±0.25*	5.06±0.21**

3.5. Histopathology study

To confirm the obtained results, we performed a histological study of the changes in rat colon under the influence of 1,2-DMH and after the use of hosta lancifolia extract.

When studying the state of animals' colon affected by 1,2-DMH, we found that the relief of the intestinal mucosa is caused only by deep crypts that reach the muscularis. Its surface was covered with prismatic epithelium. Crypts contained numerous goblet cells. Undifferentiated cells were present at the bottom of the crypts. The own lamina of the mucous membrane was represented by smooth myocytes and contained a large number of cells, the main part of which was lymphocytes. It formed folds together with the mucous membrane (Photo 1).

Microscopically, all structural components were clearly differentiated in the wall of the rats' colon in the control group. The serous membrane covered the external intermittent muscle layer with a thin, even layer. The inner circular layer formed by smooth myocytes appeared solid and more compact. Connective tissue layers of uneven thickness – loosened in some areas. Lymphocytes were also found among the connective tissue cells. Submucosa and mucosa formed folds. It was in these intestinal membranes that we noted the most pronounced structural changes. A pattern of acute inflammation was observed over a long distance or in individual areas. Dystrophically and necrotically changed covering epitheliocytes were exfoliated (Photo 2).

In the intestinal mucosa of rats that received the hosta extract, attention was drawn to increased desquamation of covering epitheliocytes with the formation of small erosions, which gave an uneven relief to the mucous membrane surface. Edema and full blood were detected in the lamina propria and in the submucosal base (Photo 3).

Focal capillary hemorrhage was also observed in the mucous membrane without hemorrhages. Visually, the number of interepithelial lymphocytes did not change compared to the control pathology, but the number of goblet cells and endocrinocytes increased. The number of undifferentiated cells increased and figures of mitoses appeared at the bottom of the crypts The lamina propria is rich in lymphocytes with an admixture of plasma cells and eosinophils. The muscular shell is loose in some places.

We observed the stabilization of structural changes with the use of hosta lancifolia during the histological examination of the rats' colon at this time. Positive dynamics were detected only in certain small areas of the mucous membrane. Enhanced desquamation of covering prismatic epitheliocytes remained, which peeled off in groups or plates, forming a thin strip on the surface. In case of uneven blood supply, venous congestion prevailed in the own lamina of the mucous membrane. The cellular composition remained at the same level

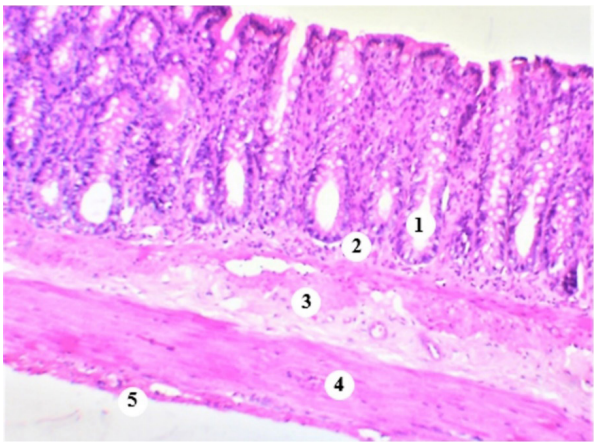

Photo 1: *Histological structure of the colon of an intact rat. 1 - crypts of the mucous membrane with goblet cells, 2 - own lamina of the mucous membrane, 3 - submucosal base, 4 - muscle membrane, 5 - serous membrane. Staining with hematoxylin and eosin. ×100.*

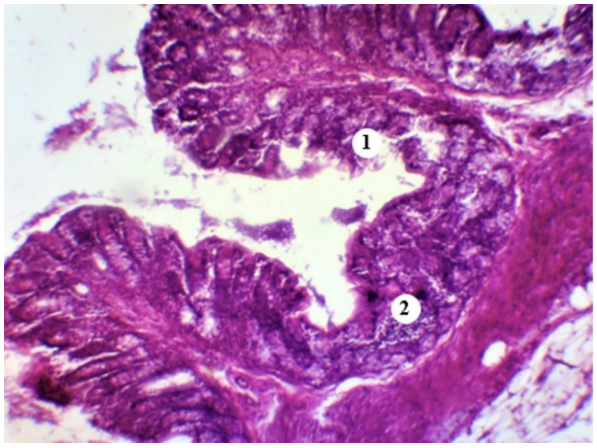

Photo 2: *Well-defined folds with foci of superficial and deep desquamation (1, 2). Staining with hematoxylin and eosin. ×40. A fragment of the colon wall of a control pathology group rat.*

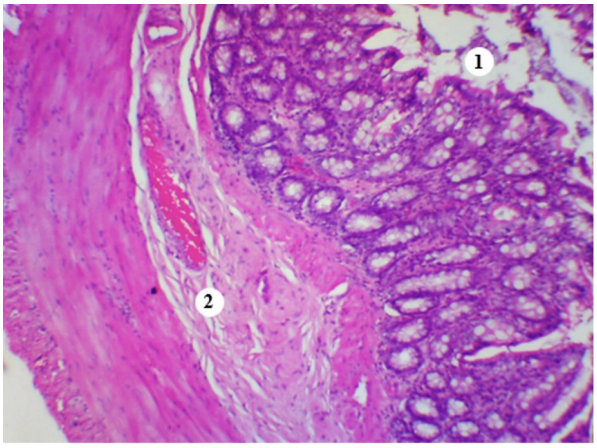

Photo 3: *Apical desquamation of the mucous membrane (1), venous congestion and edema in the submucosa (2). Staining with hematoxylin and eosin. ×100. Rat colon 5 months after administration of hosta extract.*

Photo 4: *Desquamation of the covering prismatic epithelium (1) and increased lymphocytic infiltration in the surface layer of the mucous membrane (2), thickening of the lamina propria and the submucosal base due to the proliferation of collagen fibers (3). Staining with hematoxylin and eosin. ×100. Rat colon after 7 months correction with hosta lancifolia.*

as in the previous term. However, an increase in lymphocytic infiltration of the surface layer of the mucous membrane and sclerotic changes were noted – the proportion of the connective tissue component in the lamina propria, submucosal base and muscular shell increased (Photo 4).

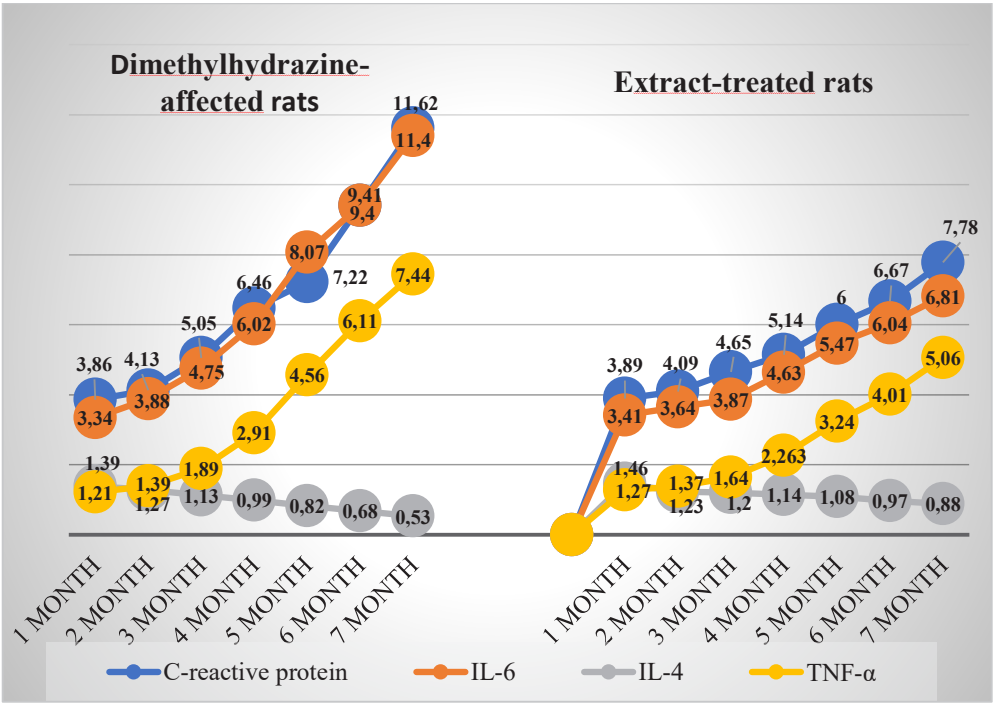

Figure 1: *Influence of hosta lancifolia leaves extract on the level of IL-4, IL-6, TNF-α and content of C-reactive in the blood serum of rats affected by 1-2-DMH and after the use of hosta lancifolia leaves extract*

4. Discussion

A significant ($p < 0.001$) increase in the level of CRP, TNF-α and IL-6 was observed during the 7-month study of chemically induced carcinogenesis against the background of a decrease in the level of pro-inflammatory IL-4 in the blood serum of animals receiving 1,2-DMH, indicating a development of the systemic inflammatory process (Fig. 1).

Cytokines are molecules of local action, synthesized in parallel, have the ability to induce each other's production and provide numerous inflammatory effects. IL-6 is synthesized in the body due to the development of inflammatory processes, during bacterial infections, under the influence of stressogenic factors and injuries. IL-6 changes the functions of the immune and endocrine systems of the body, regulating the mechanisms of recovery and activation of immune defense. IL-6 is a marker of acute systemic inflammation, and excessive production of this cytokine leads to tissue damage due to the development of an autoimmune response [20, 21].

IL-4 increases cytotoxicity of macrophages, migration of neutrophils to the inflammatory zone. It exhibits anti-inflammatory activity, as it is able to suppress the secretion of pro-inflammatory cytokines by macrophages – IL-1, IL-6, TNFα. Literature data indicate its antitumor effect, as well as cytotoxic activity against some bacteria and protozoan parasites [26-28].

TNF-α is involved in the processes of destruction and repair, because it causes the growth of fibroblasts and stimulates angiogenesis, suppresses the synthesis of collagen and proteoglycans. In addition, TNF-α increases the permeability of blood vessels, enhances chemotaxis and migration of leukocytes to the focus of inflammation, activates the enzymatic action of neutrophilic leukocytes, which ultimately leads to a cytotoxic effect [12, 25].

The use of HLLDE caused normalization of the cytokine profile in the blood serum of affected animals, which was manifested by a decrease in the content

of pro-inflammatory cytokines IL-6, TNF-α and an increase in the content of anti-inflammatory cytokine IL-4 (Fig. 1). The studied extract also had a positive effect on the level of CRP in the blood serum of rats with simulated oncopathology. A probable decrease in this indicator was observed already after 3 months of HLLDE daily administration (Fig. 1).

Data obtained experimentally indicate the anti-inflammatory and oncoprotective effect of the extract from hosta lancifolia leaves. The conducted study demonstrate importance of the cytokine link in the regulation of inflammatory processes in the pathogenesis of chronic oncology and importance of the pro- and anti-inflammatory cytokines level determining, as well as the level of CRP in animal blood serum as a criterion for clinical course of the disease and the effectiveness of oncology treatment.

5. Conclusions

Results of the research prove that in animals affected by 1,2-dimethylhydrazine, there is an imbalance in the content of pro- and anti-inflammatory cytokines in blood serum – a probable increase in the level of pro-inflammatory cytokines (TNF-α, IL-6) against the background of a decrease in the content of anti-inflammatory cytokine IL -4, as well as an increase in the level of C-reactive protein.

Due to the rich content of biologically active substances, hosta lancifolia extract showed anti-inflammatory and oncoprotective effects, significantly reducing the content of C-reactive protein, pro-inflammatory cytokines IL-6 and TNF-α, increasing the content of anti-inflammatory cytokine IL-4 in the blood serum of rats with chemically induced oncology.

Experimental work creates prospects for further study of dry extract from hosta leaves to create a medical drug based on it with anti-inflammatory, oncoprotective action, effective in the prevention or complex therapy of cancer diseases.

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Conflicts of interest: *The authors report no financial or any other conflicts of interest in this work.*

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ΕΚΔΗΛΩΣΕΙΣ - MEETINGS

• MARCH 9-11, 2023, PATRAS, GREECE
19th Hellenic Symposium on Medicinal Chemistry (HSMC-19)
https://helmedchem2023.gr

• JUNE 10-13, 2023 | BOSTON, MA, UNITED STATES
ACSMEDI | EFMC Medicinal Chemistry Frontiers 2023 - Joint Symposium on Medicinal Chemistry
https://www.medchemfrontiers.org/

• JUNE 11-14, 2023, IOANNINA, GREECE
16TH INTERNATIONAL SYMPOSIUM ON APPLIED BIOINORGANIC CHEMISTRY
https://isabc2023.com/registration/

• JULY 2-6 2023 ANKARA, TURKEY
33rd International Symposium on Pharmaceutical and Biomedical Analysis
https://pba2023.com/

• JULY 5-7, 2023 LILLE, FRANCE
57th edition of the International Conference on Medicinal Chemistry (RICT 2023).
https://www.rict2023.org/

• JULY 16 – 18 2023 THESSALONIKI, GREECE
XII Meeting of the Paul Ehrlich PhD NetWork in Medicinal Chemistry
https://medchem2023.com

• SEPTEMBER 4-6, 2023, BELGRADE, SERBIA
IAPC-10 Meeting
Physico Chemical Methods in Drug Discovery and Development
And ADMET and DMPK
https://iapchem.org/index.php/iapc-10-home

• SEPTEMBER 3-7, 2023 | ZAGREB, CROATIA
IX EFMC International Symposium on Advances in Synthetic and Medicinal Chemistry
https://www.efmc-asmc.org/

• SEPTEMBER 7-8, 2023 | ZAGREB, CROATIA
EFMC-YMCS 2023
10th EFMC Young Medicinal Chemists' Symposium | Anniversary Edition
https://www.efmc-ymcs.org/

• SEPTEMBER 17-20, 2023 | CHANIA, CRETE
13th International Conference on Instrumental Methods of Analysis: Modern Trends and Applications, IMA 2023
http://aclab.web.auth.gr/ima2023/

• NOVEMBER 16-18, 2023 | BASEL, SWITZERLAND.
International Symposium on Chemical Biology (EFMC-ISCB)
https://www.efmc-iscb.org/