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Professional paper

THE EFFECT OF SHARRI MOUNTAIN TEAS ON DIFFERENT STAGES OF EMBRYONIC DEVELOPMENT IN QUAIL AND CHICKEN EGGS

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Abstract

The use of these teas is known even in ancient times by the local population, but recently there are data on the medicinal character of these teas as a form of herbal supplements with a healing character.
The effect of the extract of these teas has been studied on quail eggs Coturnix japonica, and chicken eggs Gallus domestica, by applying extract with a certain dose to quail and chicken eggs, at different stages of embryonic development during ontogenesis.
The dose given in extract form has resulted in macroscopic histological changes and up to the phenomenon of teratogenesis during application at certain stages of embryonic development.
This research aims to explore the effect of the given dose of plant extracts on certain stages of embryo development during ontogenesis and to see the benefits or consequences of taking the dose in a certain amount and at a certain time to observe these macroscopic changes histopathology.

Introduction

Sharr Mountain massif is considered one of the largest mountain ranges with special geographical characteristics and an expressed biological diversity, with a special endemism and a wide spectrum of flora and fauna characteristic of this mountain massif.

Also, Sharr Mountain is characterized by plant species of special importance and variety, both from the presence and the spectrum of the biological character, but also from the spectrum of the nutritional and health character. In the context of teas with nutritional character and health benefits, we can count species such as: mint (Mentha piperita), St. John's wort (Hypericum perforatum), sideritis (Sideritis scardica), and chamomile (Matricaria camomila), etc.

These mountain products can be considered as bioproducts of particular importance both in terms of presence, quality and health benefits, therefore the exploration of these species would have a positive cost for consumption as a form of teas or plant extracts for commercial purposes but also for health benefits and above all for the population of the country, the wider region (Rehault-Godbert S, GuyotN, Nys Y, 2019).

The use of these teas is known even in ancient times by the local population, but recently there are data on the medicinal character of these teas as a form of herbal supplements with a healing character.

The effect of the extract of these teas has been studied on quail eggs Coturnix japonica, and on chicken eggs Gallus domestica, applying extract with a certain dose to quail and chicken eggs, at different stages of embryonic development during ontogenesis (Shabani L, Beadini. N, Beadini Sh, 2022).

The dose given in extract form has resulted in macroscopic histopathological changes up to the phenomenon of teratogenesis during application at certain stages with certain doses of embryonic development.

This research aims to explore the effect of the given dose of plant extracts on certain stages of embryo development during ontogenesis and to see the benefits or consequences of taking the dose in a certain

amount and at a certain time to observe macroscopic histo changes pathological.

The purpose of the study

This paper aims to investigate the effect of the herbal extract of mint (Mentha piperita), St. John's wort (Hypericum perforatum), sideritis (Sideritis scardica), and chamomile (Matricaria camomila) teas, applying the extract at a certain dose to quail eggs and chicken, at different stages of embryonic development during ontogenesis.
The dose given in extract form has resulted in macroscopic histological changes and up to the phenomenon of teratogenesis during application at certain stages of embryonic development.
This research aims to explore the effect of the given dose of plant extracts on certain stages of embryo development during ontogenesis and to see the benefits or consequences of taking the dose in a certain amount and at a certain time to observe these macroscopic changes histopathology.

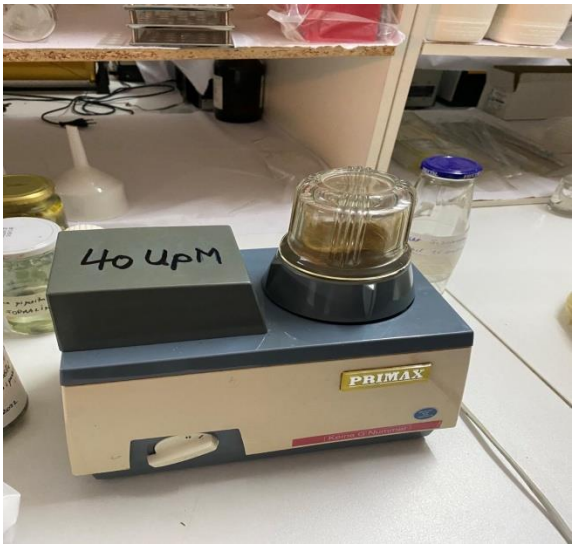


Figure 1. Apparatus for grinding tea

Figure 2. Apparatus for extracting teas

For the extraction of teas (Menta piperita), St. John's wort (Hypericum perforatum), sideritis (Sideritis scardica), and chamomile (Matricaria camomila) the Rota Bucher apparatus was used, which made the extraction of teas in a liquid state as plant extract fig1, 2.
The extract was filtered through a funnel with special sieves and stored in separate jars where they were preserved and prepared in certain concentrations as separate doses fig. 3,4.

Figure 3. Funnel and extraction funnel

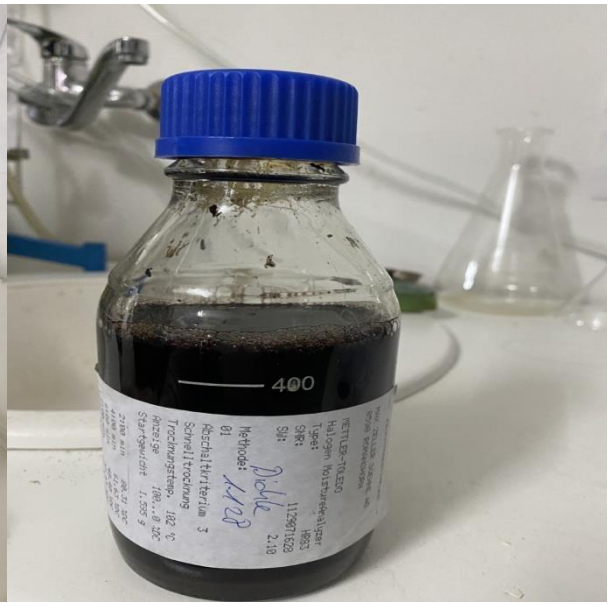


Figure 4. Herbal extract shaving nozzle

The plant specimens were collected in Sharr Mountain massif of at an altitude of 1700 m above sea level in the time period: July-September 2021/2022 and were transferred to the drying chamber according to the drying procedure fig. 5,6,7,8.

Figure 5. St. John's worth (Hypericum perforatum) - 1700 m

Figure 6. Mint (Menta piperita) -1700m

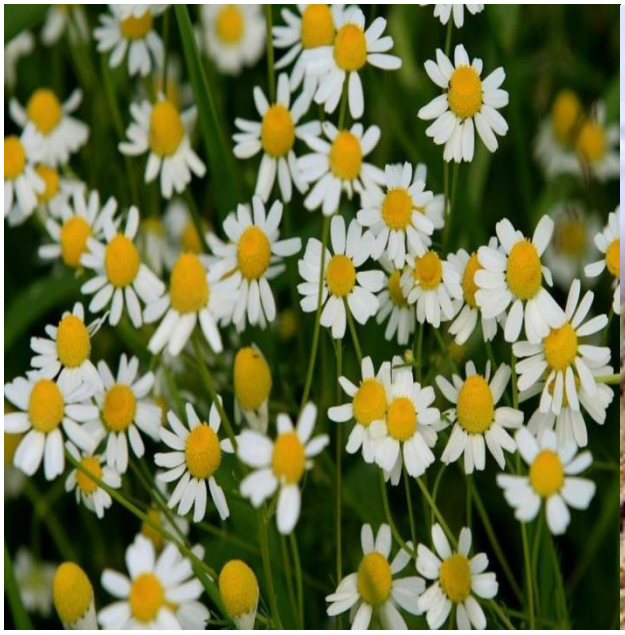


Figure 7. Chamomile (Matricaria camomila) - 1500m

Figure 8. Sideritis (Sideritis scardica) - 1800m

Initially, quail and chicken eggs were checked with a fluorescent lamp to see if they are fertilized or sterile, the sterile eggs were removed and then the fertilized ones were dosed according to the dosing procedure (see procedure) fig. 9,10.
The eggs were previously measured and placed in a special Incubator for incubating chicken eggs and quail eggs at a temperature of 37.5 0C and a humidity of 70%.

Figure 9. Fertilized and marked eggs

Figure 10. Dosing procedure

The eggs were previously measured and placed in a special Incubator for incubating chicken eggs fig 11 and quail eggs fig 12, at a temperature of 37.5 0C and a humidity of 70%.

Figure 11. Marked eggs in the incubator holder

Figure 12. Incubator for incubating chicken eggs

Figure 13. Incubator for incubating chicken eggs

Figure 14. Incubator for incubating quail eggs

On the fourth day after incubation, a measurement was made once again to see the change during the incubation phase, and then they were given the dose set for each species separately fig 13,14.
The dose of herbal extract was given to you in a certain position of the eggs, specifically in the air chamber, in order to absorb the extract as best as possible through the membrane - the serous envelope of the egg.
Incubation lasted 17 days for chicken eggs and 21 days for chicken eggs.
During the incubation phase, certain stages of embryonic development were monitored and any changes in certain stages were analyzed until the end of incubation.
After hatching, the specimens are placed in special jars to be preserved with methanol or 70% alcohol for the purpose of researching macroscopic changes or other histopathological or teratogenic changes fig. 15,16.

Figure 15. Decapitation of the fetus **Figure 16.** Preservation of the fetus in conical vials with 70% alcohol

Research results

Measurement parameters such as: marking, measurement before incubation, measurement after incubation, control eggs, experimental eggs, morphological changes were investigated in quail and chicken eggs.
In fig. 17 and 18 show the sequence of eggs according to the marking procedure and in fig 18 the eggs are opened to be explored according to certain stages of embryonic development.

Figure 17. Quail eggs lined up for research **Figure 18.** Quail eggs during the research stage

In Fig 19 and 20 chicks are shown after 21 days of incubation and acclimatized for.

Figure 19. Acclimatized chick after the 21st day of incubation

Figure 20. Chicken chicks acclimatized to normal life

Figure 21 shows the chicks placed according to the position as control birds in certain and marked positions.

Figure 21. Chicks placed in certain positions according to hatching after incubation

Comparison of control and experimental rats treated with Chamomile (Matricaria camomila) and Sideritis (Sideritis scardica) teas with a normal dose of regular tea.

From the results of the research, it is clear that the usual mixed dose of Chamomile (Matricaria camomila) and Sideritis (Sideritis scardica) teas has resulted positively in normal stages of embryonic development in the chicken bird. From the results of the research, it can be seen that in the control eggs from the first day of

incubation to the 21st day, as long as the incubation normally lasts in chicken eggs, the stages of development and the trend of the ontogenesis were analyzed fig 19, 20, 21 .
The results of the research on the experimental eggs treated with a mixture of Chamomile (Matricaria camomila) and Sideritis (Sideritis scardica) teas have resulted positively and have an almost similar performance to the control eggs, which means that the given dose of Chamomile (Matricaria camomila) and Sideritis teas (Sideritis scardica) has somehow stimulated the ontogenic stages of embryo development in chicken eggs (see the following figures).

Figure 22 a) First day of incubation - control egg

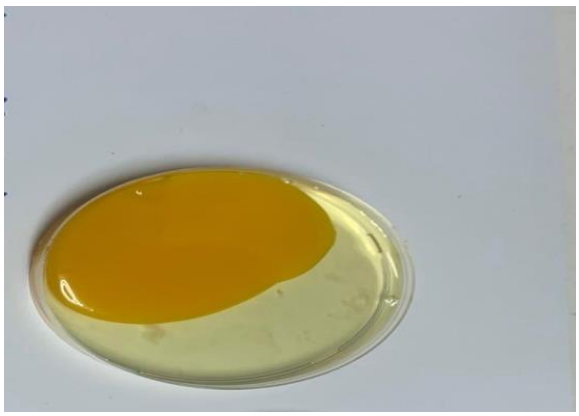


Figure 22b) First day of incubation - experimental egg

In figure 22 a, it is clearly observed that the egg is fertilized and the differentiation in blastomeres is observed, as in the control egg, in which the same situation is observed, figure 22 b.

Figure 23 a) First day of incubation - control egg

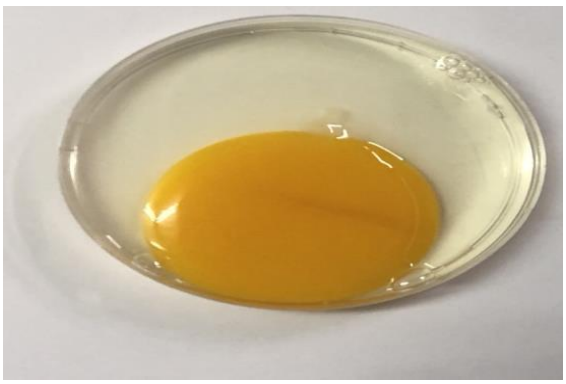


Figure 23 b) First day of incubation - experimental egg

In figure 23 a, the differentiation in blastomeres is clearly visible, while in the experimental egg, the differentiation in 23 b blastomeres and the expressed and advanced furrowing 23 b can be clearly observed.

Figure 24 a) Second day of incubation - control egg **Figure 24** b) Second day of incubation - experimental egg

In figure 24 a, the differentiation into blastomeres is clearly visible, while in figure 24 b, the experimental egg, the differentiation into 2 blastomeres and the expressed and advanced furrowing are clearly visible.

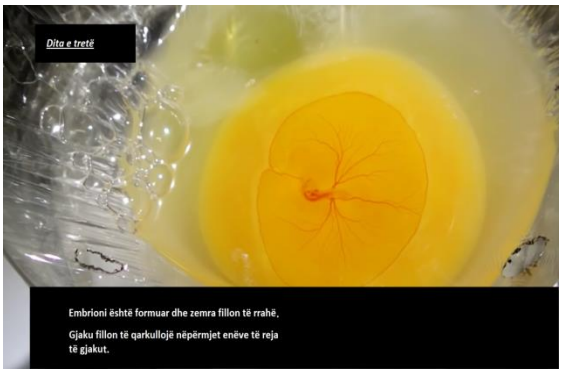


Figure 25 a) Third day of incubation - control egg **Figure 25** b) Third day of incubation - experimental egg

In figure 25 a, the differentiation of the heart and blood vessels is clearly visible, whereas in the experimental egg, whereas in the experimental egg, there is stagnation of differentiation fig. 25 b.

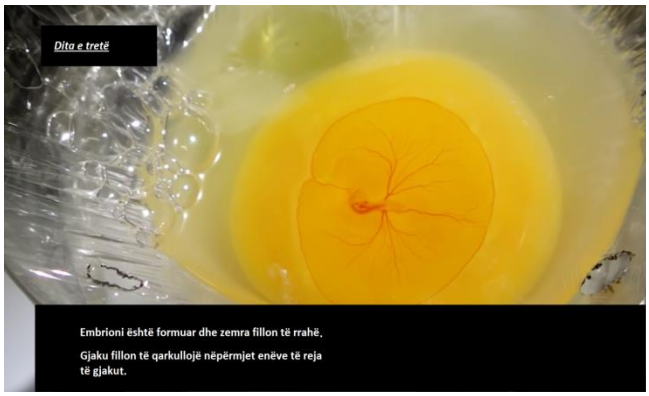


Figure 26 a) Third day of incubation - control egg **Figure 26** b) Third day of incubation - experimental egg

In figure 26 a, the differentiation and pulsation of the differentiated heart and blood vessels are clearly visible, while in figure 26 b, in the experimental egg, there is stagnation of differentiation.

Figure 27 a) Fourth day of incubation - control egg

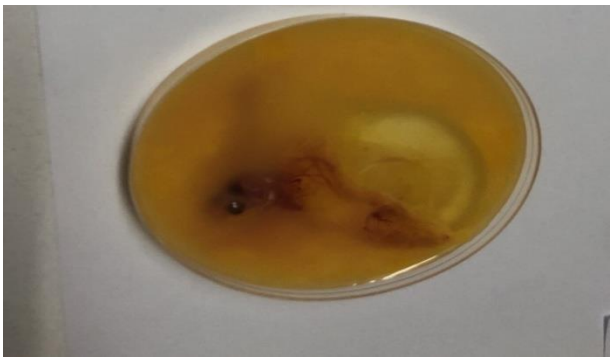


Figure 27 b) Fourth day of incubation - experimental egg

In figure 27 a, the differentiation and pulsation of the heart is clearly visible, the amniotic membrane grows, the shape of the embryo is clearly visible and the differentiation of the cephalus and the differentiation of the eyes and blood vessels are well differentiated, while in figure 27 b, in the experimental egg, we have a more advanced differentiation of these bodies.

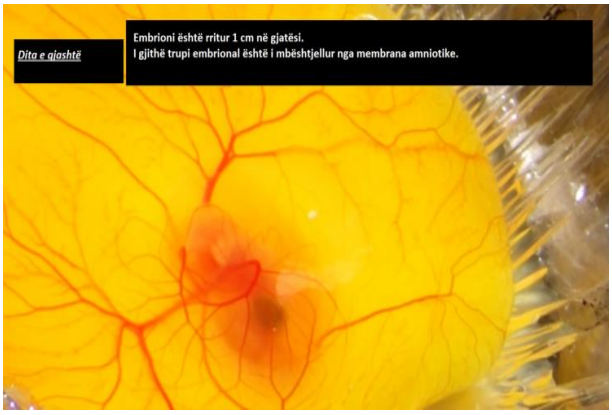


Figure 28 a) Sixth day of incubation - control egg

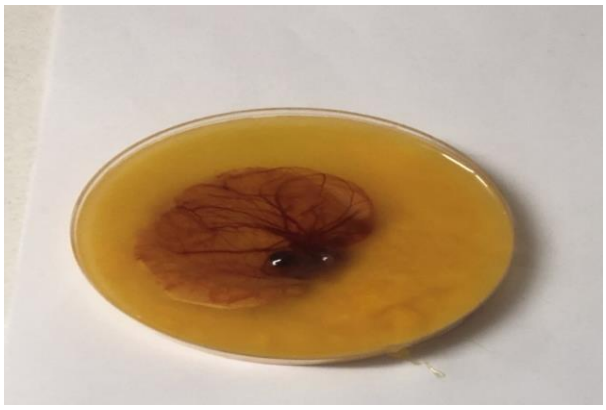


Figure 28 b) Sixth day of incubation - experimental egg

In figure 28 a, the differentiation of the heart and the expressed vascularization of blood vessels are clearly visible, the amniotic membrane grows and envelops the fetus, while in figure 28 b, in the experimental egg, we have a more advanced differentiation of these organs.

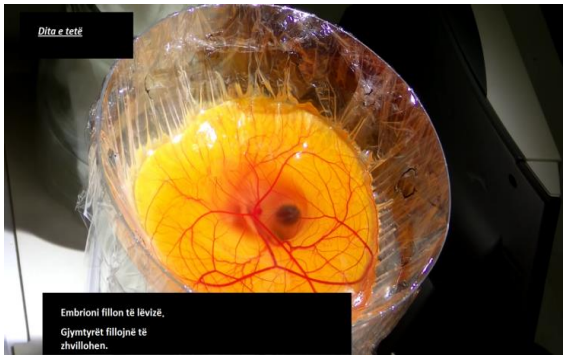


Figure 29 a) Eighth day of incubation - control egg

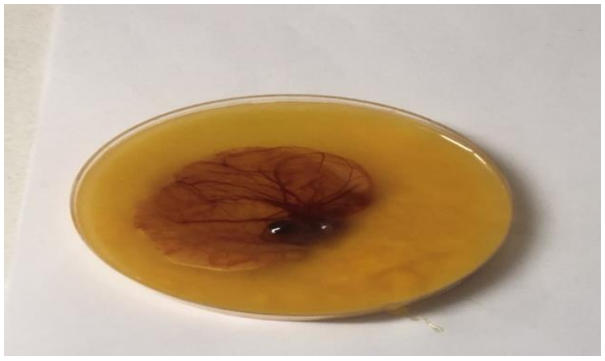
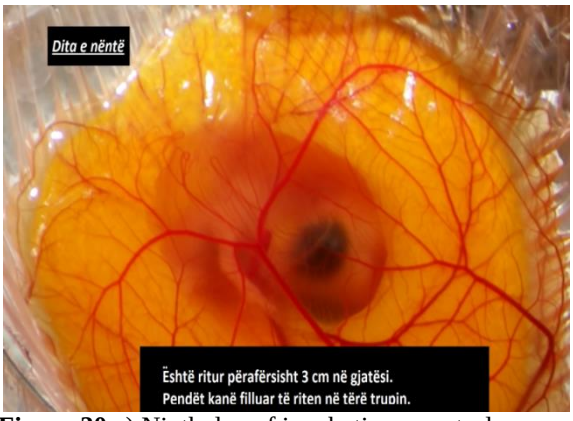


Figure 29 b) Eighth day of incubation - experimental egg

Figure 29 a clearly shows the differentiation of the heart and the expressed vascularization of the blood vessels, the amniotic membrane grows and envelops the fetus and the embryo begins to move, while in the experimental egg we have a more advanced differentiation of these organs figure 29 b.



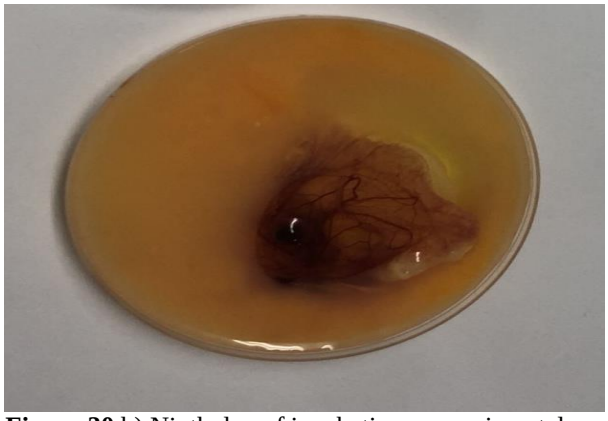
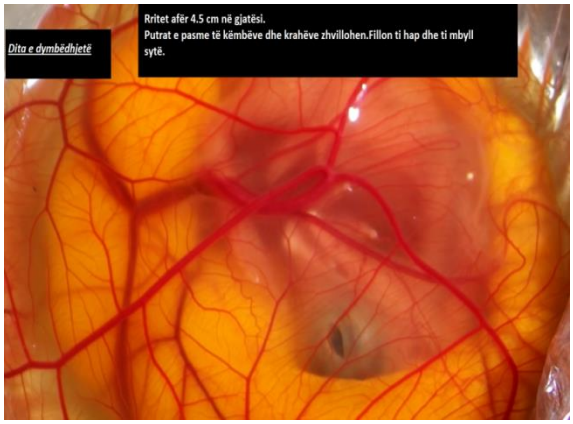


Figure 30 a) Ninth day of incubation - control egg **Figure 30** b) Ninth day of incubation - experimental egg

In figure 30 a, the differentiation of the fetus is clearly visible at approximately 3 cm and the embryo begins to move, and we also have the differentiation of the extremities, while in figure 30 b, in the experimental egg, we have a more advanced



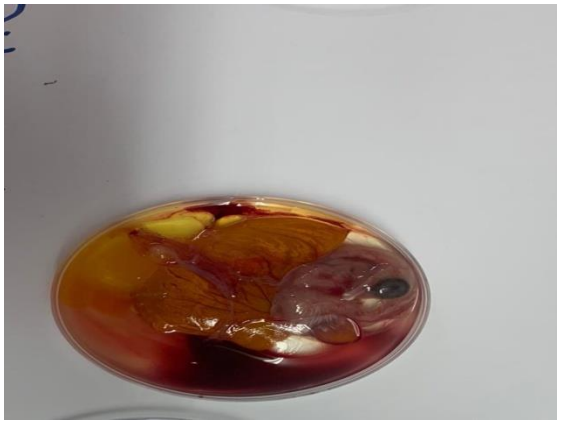


Figure 31 a) Twelfth day of incubation - control egg **Figure31** b) Twelfth day of incubation - experimental egg

Figure 31a clearly shows the differentiation of the fetus approximately 3 cm and the embryo begins to move and we also have the differentiation of the extremities, while in the experimental egg we have a more advanced differentiation of these organs fig 31b.

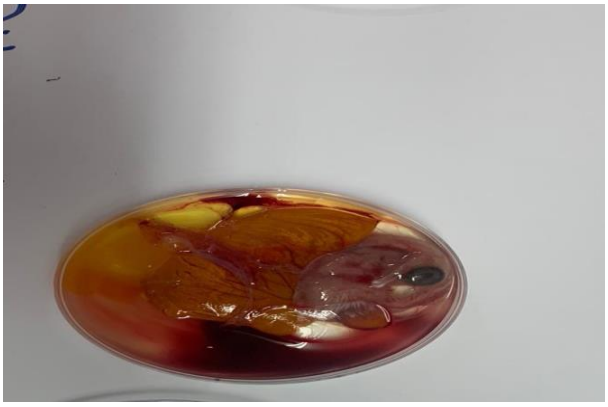


Figure 32 a) Fourteenth day of incubation - control egg **Figure** 32 b) Fourteenth day of incubation-experimental egg

Figure 32 a clearly shows the differentiation of the fetus in even more advanced stages, we also have the differentiation of the extremities and other organs, while in the experimental egg we have a more advanced differentiation of these organs, as can be seen the differentiation of the head, body and extremities fig 32 b.

Figure 33 a) Fifteenth day of incubation - control egg **Figure 33** b) Fifteenth day of incubation - experimental egg

Figure 33 a clearly shows the differentiation of the fetus in size 7 cm and the reduced vitellus in even more advanced stages, we also have the differentiation of the extremities and other organs, while in the experimental egg we have a more advanced differentiation of these organs, as can be seen the differentiation of the head, body and extremities fig. 33 b.

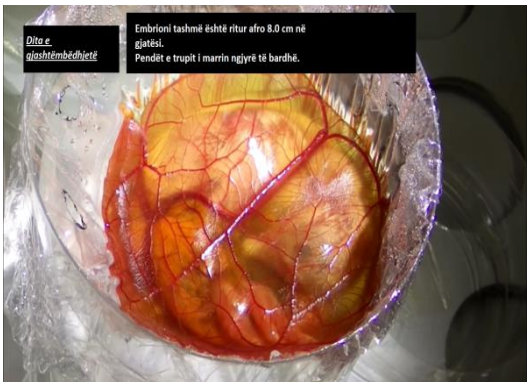


Figure 34 a) Sixteenth day of incubation - control egg **Figure 34** b) Sixteenth day of incubation - experimental egg

In figure 34 a, the differentiation of the fetus is clearly visible in the size of 8 cm and the greatly reduced vitelline in even more advanced stages, we also have the differentiation of the extremities and other organs, while in the experimental egg we have a more advanced differentiation of these organs, as can be seen the differentiation of the head body and extremities fig 34 b.

Figure 35 a) Nineteenth day of incubation - control egg

Figure 35 b) Nineteenth day of incubation - experimental egg

In figure 35 a, the differentiation of the fetus in size 10 cm and very reduced amnion is clearly seen in even more advanced stages, we also have the differentiation of the extremities and other organs, while in figure 35 b, in the experimental egg, we have a more advanced differentiation of these organs, as can be seen the differentiation of head, body and extremities.

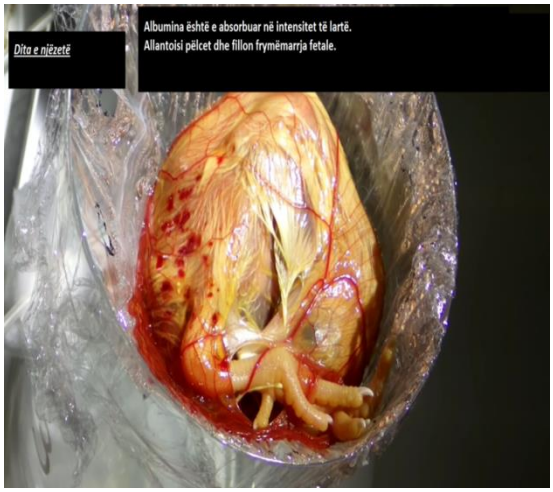


Figure 36 a) Twentieth day of incubation - control egg

Figure 36 b) Twentieth day of incubation - experimental egg

In figure 36 a, the differentiation of the fetus in size 10 cm and a very reduced amnion and allantois cleft, the organs in even more advanced stages, we also have the differentiation of the extremities and other organs, while in figure 36 b, in the experimental egg, we have a more advanced differentiation of these organs as the differentiation of the head, body and extremities is observed.

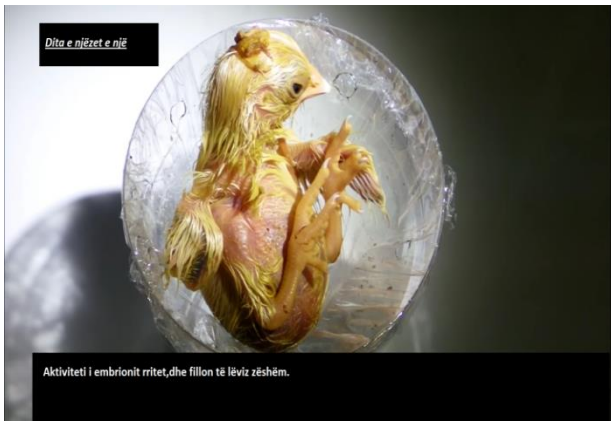


Figure 37 a) Twenty-first day of incubation - control egg **Figure 37** b) Twenty-first day of incubation - experimental egg

In figure 37 a, it is clear that the activity of the fetus increases and an accelerated movement begins, as in the experimental egg figure 37 b.

Figure 38 a) Twenty-second day of incubation - control egg **Figure 38** b) Twenty-second day of incubation - experimental egg

In figure 38 a, the activity of the bird in normal environmental conditions is clearly visible, as in the experimental eggs, figure 38 b.

Time stages of the incubation period of chicken eggs in the period of 21 days and macroscopic examination.

From figs 39 a and 39 b it is clearly observed that the eggs of chicken (Galus domestica) treated with a dose of 10/20 ml of tea extract mint (Menta piperita), St. John's wort (Hypericum perforatum) which were found to have stagnated and failed during the development stages embryonic. From the figure it is clear that in almost all experimental eggs we have no embryonic development and no differentiation in certain stages such as differentiation in blastomeres, differentiation in the morula stage, blastula and gastrulation, which means that we have no ontogenic development and stagnation or non-differentiation of organs during organogenesis. This could be a consequence of a high dose or an overdose, but this phenomenon still needs to be explored.

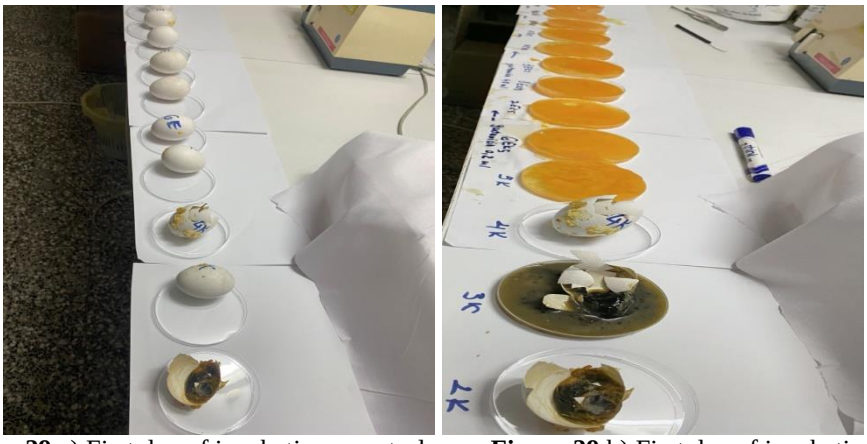


Figure 39 a) First day of incubation - control egg **Figure 39** b) First day of incubation - experimental egg

From fig 40 a and 40 b, it is clearly observed that the eggs of quail (Coturnix japonica) treated with a dose of 0.3 ml of tea extract mint (Menta piperita), St. John's wort (Hypericum perforatum) have stagnated and failed during the stages of embryonic development. From the figure, it is clearly seen that in the control eggs certain stages of embryonic development have been developed from fertilization to the fetal check, while in almost all the experimental eggs we have no embryonic development nor differentiation in certain stages such as differentiation into blastomeres, differentiation into the morula, blastula and gastrullation stage, which means that we do not have ontogenic development and stagnation or non-differentiation of organs until organogenesis. From the figure it is also clear that in some cases we have differentiation up to organogenesis but we do not have complete hatching and stagnation at this stage that this phenomenon is still being studied 40a, 40b.

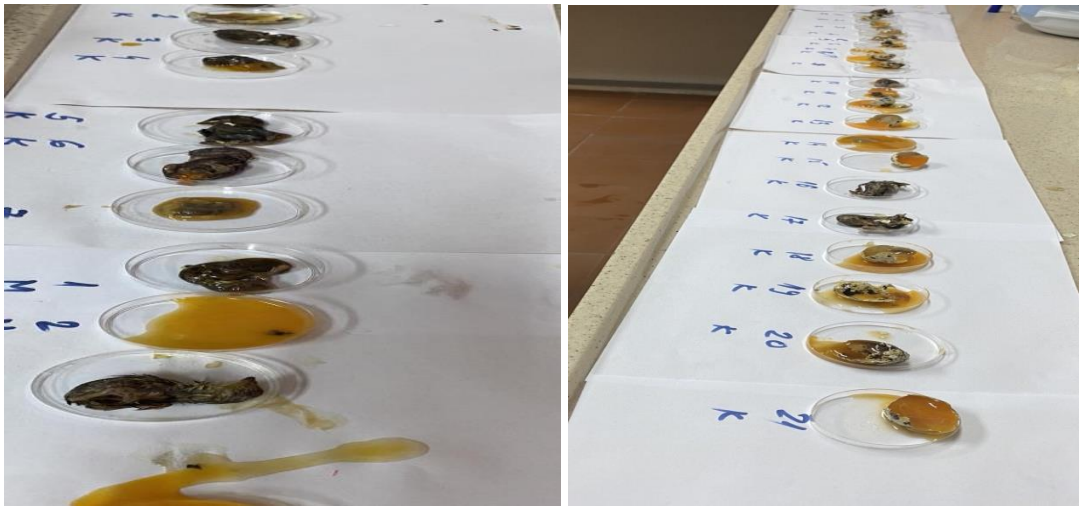


Figure 40 a) First day of incubation - control egg **Figure 40**b) First day of incubation - experimental egg

Figure 41 a) First day of incubation - control egg

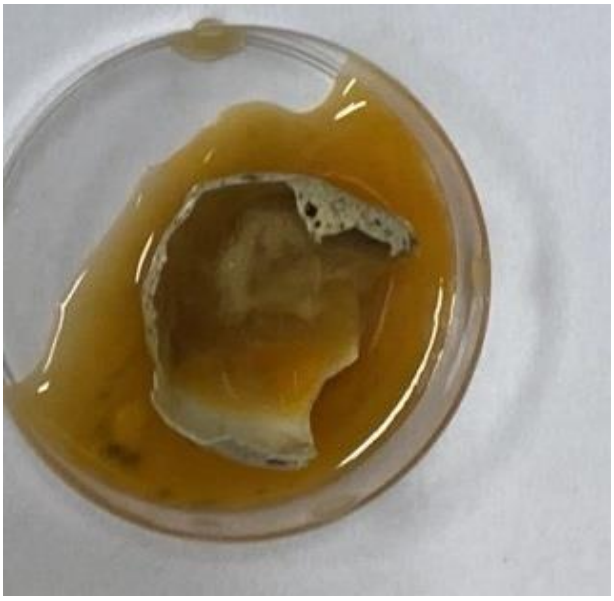


Figure 41b) First day of incubation - experimental egg

In figure 41a, the differentiation in blastomeres is clearly visible, while in the experimental egg, the differentiation in blastomeres and furrowing is not clearly observed at all, and it is observed that we have a heterogeneous mass of the yolk, fig 41b.

Figure 42 a) Tenth day of incubation - control egg

Figure 42 b) Twelfth day of incubation - experimental egg

Figures 42 a and 42b clearly show the differentiation of the organs and the cracking of the shell to get the bird out into the open environment.

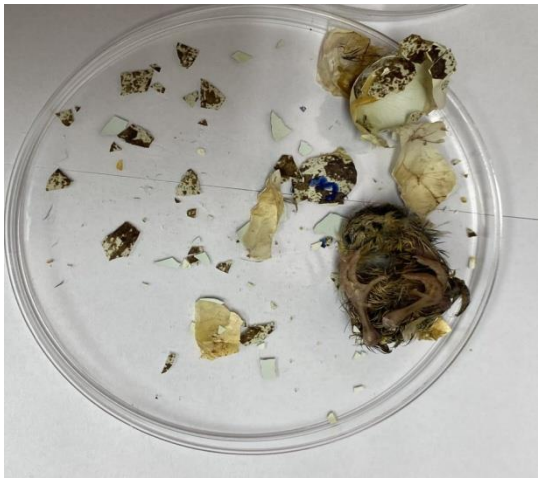



Figure 43 a) Fifteenth day of incubation - control egg **Figure 43** b) Sixteenth day of incubation - control egg

Figures 43 a and 43 b clearly show the differentiation of the organs and the cracking of the shell to get the bird out into the open environment.


Figure 44 a) Seventeenth day of incubation - control egg **Figure 44** b) Eighteenth day of incubation - experimental egg

In figures 44 a and 44 b, the differentiation of the body and the crack are clearly visible, and the bird is more in the open environment and lives independently.

Discussion

Bioproducts and natural products have a positive impact on the quality of organic foods because they are qualitative and qualitative in terms of chemical composition and organic compounds, therefore their use in the form of plant extract gives positive effects both in terms of quality and biological health (Uchechukwu Edna Obianwuna, Vivian U. Oleforuh-Okoleh, Jing Wang, Hal-Jun Zhang, Guang- Hal Qi, Kai Qiau and Shu-Geng Wu, 2021).

This phenomenon is also similar to the results of our several years of research, which show that the use of plant extracts from bioproducts gives positive effects on the quality of food, both in terms of nutritional character and the character of organic ingredients, which speaks of components with high nutritional values

and qualities (Shabani L, Beadini. N, Beadini Sh, 2022, Sharma MK, Dinh PA, 2020, Kovacs-Nolan J, Phillips M, Mine Y, 2005, Chang X, Qiu K,Wang I, Zhang H, You S, MI S et al, 2021).

However, the lethal and non-proportional dose has shown a negative effect on the stages of embryonic development and has especially reflected complete partial stagnation in certain stages of embryonic development and has also shown histopathological changes in certain organs and has provoked teratogenesis, which they found it in the dose and the dose effect (Ritu Singroha, S.K Srivastava, Pankaj Chhikara, 2013).

Recently, herbal products find use among the human population and are used in different forms such as teas, creams, herbal therapy, as melmesa in food and other forms.

This has also led to thinking about herbal supplements such as mint teas (Mentha piperita), St. John's wort (Hypericum perforatum), sideritis (Sideritis scardica), and chamomile (Matricaria camomila) which find an application as an alternative therapy in the population suffering from different diseases.

Mint (Mentha piperita L.) - It is found in gardens and wet places, mostly cultivated, but very rarely in places where it is not cultivated. Description: It has a length of 40-110 cm, the rhizomes are quite developed, it has toothed oval leaves, and the flowers are violet in color. Harvesting: During the summer, the leaves are harvested (several times), while at the time of the plant's flowering, the whole plant, together with leaves and flowers. It is used against the flu, for the healing of the respiratory and digestive organs, as well as against stomach cramps.

Chamomile (Matricaria chamomila)- It is used against pains and contractions, inflammations of the skin and mucous membrane of the mouth, throat, nose, eyes, as well as for regulating food digestion, cleaning the urinary tract from the outside and inside, against hemorrhoids.

Chamomile is used as a tonic, stomachic, anodyne, antispasmodic, laxative, diaphoretic, analgesic, carminative, anti-inflammatory, sedative.

St. John's wort (Hypericum perforatum) - German studies have shown that this plant has powerful antidepressant effects and can be used in cases of moderate to severe depression.

Researchers have compared the effect of pansy flower with the effect of paroxetine, one of the most popular drugs for the treatment of depression.

The dry herb of lulebasan serves as an antidepressant (hypericin), antiseptic, anti-wrinkle, heals wounds, softens swelling, promotes the flow of bile from the gall bladder, etc. It inhibits the development and growth of microbes (antiseptic) in wounds, pimples and burns. In this case, rosemary oil is recommended for external use. In addition, used orally, it is effective in metabolic processes in diseases of the digestive system, liver, gall bladder, hemorrhages in the digestive system and lungs, in insomnia and in kidney diseases. Improves blood circulation and calms conditions with nervous tension. Our folk medicine advises its use for the treatment of gastritis and ulcers of the stomach and duodenum.

Sideritis mountain tea (Sideritis scradica) - This tea grows on the tops of the mountains, especially in the Galicica Mountain, at an altitude of 2000 m. This mountainous space is declared a national park, where all types of tea are protected by law. The plant has a characteristic growth where it grows in plant communities in mounds around the stones of the mountain slopes, the plant grows by being exposed to the sun all day. The roots are powerful, very deep, to provide water. The plant grows in very dry places, it is popularly known as popular tea, mountain saw tea.

Chemical composition: the plant has tannins, flavonoids, phenols, phenylcarboxylic acids, the tea has essential oil which is characteristic of the type of siderite, the aroma is very pleasant and we can say that it is unique. The use of tea finds application for deep colds, for increasing immunity, helps prevent osteoporosis. Active elements that include diterpenoid, flavonoid.

There are data that mint (Mentha piperita), St. John's wort (Hypericum perforatum), sideritis (Sideritis scardica), and chamomile (Matricaria camomila) teas have healing effects and, on the other hand, can also

provide stimulating or inhibitory effects depending on the dose in the stage certain embryonic development of quail eggs and chicken eggs.

Conclusion

Our research is focused on the teas of the Sharr Mountain massif of such as: mint (Menta piperita), St. John's wort (Hypericum perforatum), sideritis (Sideritis scardica), and chamomile (Matricaria camomila) and their impact on the healing effect and stimulus for the organism.

There are data that mint (Menta piperita), St. John's wort (Hypericum perforatum), sideritis (Sideritis scardica), and chamomile (Matricaria camomila) teas have healing effects, but on the other hand, they can also provide stimulating or inhibitory effects depending on the dose given in certain stages of embryonic development of quail eggs and chicken eggs.

The given dose of 0.3 ml of mint and St. John's wort extract solution has provoked stagnation of processes during certain stages of embryonic development in quail and chicken eggs and in some cases even total inhibition of organogenesis.

The given dose of 0.3 ml of the scardica and chamomile tea extract solution has resulted in normal development but also in the stimulation of processes at certain stages of embryonic development in chicken eggs and expressed differentiation in organogenesis.

This phenomenon shows that not all types of teas can give positive effects at certain stages of embryonic development, therefore it is worth further researching the effect of the extract of these species because the stimulating or inhibiting effect of these teas must be proven.

This research will be able to find application in the field of alternative therapy and bio products in the food industries as a product with an effective character for the production of natural foods as a necessity for maintaining the quality of food and health in general.

These teas can also serve as alternative supplementary therapy and can significantly improve human health, therefore they can find healing applications in human health.

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