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RESEARCH ARTICLE

Studying the proliferation activity of the plant Caper (*Capparis spinosa* L.)

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Abstract

The article presents information on the methods and results of studying the influence of extracts prepared from various organs of the caper plant (*Capparis spinosa* L.), which in the Samarkand region exhibits the absence of proliferative activity of monocyte macrophages and mouse RAW 264.7. There are many scientific and research works devoted to the study of the medicinal properties of *Capparis spinose* L. cappers growing in Uzbekistan, as well as many works dedicated to identifying the molecular mechanisms of the immunomodulatory properties of the plant, which have not yet been studied. In medicine, consuming ripe fruits of *C. spinosa* L. has long been known to treat various diseases' inflammatory processes, including stomach inflammation and intestinal bleeding processes of haemorrhoids. The experiments show that concentrations of plant extracts at a concentration of 25.5 µl have a destructive effect on all samples. A concentration of 1 µl reduces the proliferative activity of cells of all plant extracts, except for seed extract; as a result, when exposed to 1 µl of seed extraction, the population level was the highest. In cells stimulated with LPS, the level of proliferation was high at a concentration of 0.5 µl of extracts of flowers, fruits and roots. Cytotoxicity was observed in extratax leaves, which may explain the antibacterial activity.

Keywords

Adhesion; *Capparis spinosa* L.; extraction; macrophage; monocyte; PBS; RAW 264.7

Introduction

Caper (*Capparis spinosa* L.) is a herbaceous, low-lying woody plant with a stem length of 200-300 cm, (1) growing in the conditions of Uzbekistan (Fig.1). Leaf length is 2-4.5 cm, green, glabrous or less pubescent; in the axils of the leaves there are rarer spines 4-7 mm long (2)—flowers from 4-5 sm to 8 sm, located singly in the leaf axils. There are four calyxes, curved-oval, with a sharp tip, green. There are four petals; the stamens are white of different lengths and glued to the side in the dust. The average weight of one fruit is 7 grams; the fruit contains up to 570 seeds, on average 350 seeds, and the seeds are inside the red pulp (3).

Caper (*Capparis spinosa* L.) seeds are kidney-shaped. The average weight of 1000 seeds is 755-760 mg (4). Caper bushes with such a morphological structure are observed in the Urgut district of the Samarkand region. Since the Urgut region is mountainous, when studying the morphological characteristics of fruits, one bush can have up to 500 fruits,

Fig. 1 Flower and fruit *Caparris spinosa L.*

its height is 5.5 cm, and its weight is up to 30 grams (5).

Caper (*Capparis spinosa* L.) is one of the most important economic species of the *Cappariadacea* (6) cultivated worldwide, especially in the Mediterranean basin and Asia. This is why Caper has many names, such as Alcaparo in Spain, Alaf-e-Mar in Persian, Cappero in Italy and wild watermelon in China (7). Caper plant blooms in July and August. The fruit is a multi-seeded berry containing about 36% lipids and 18% protein (8). They often eat fragrant flower buds, which are dried and salted in the sun before flowering. Caper is classified by size: pea-sized flower buds, smaller than olives, are tastier and more valuable. In addition to their valuable nutritional properties, capers do not contain many calories: 100 g of capers contain only 23 kcal (9).

This plant is widely studied in the field of medicine and pharmaceuticals due to its medicinal properties (10), and the polyphenols (11) are found in various organs of flowers, fruits, roots, leaves and stems (12). It is known that polyphenols are of great importance for human health. It has powerful potential in the prevention of diseases of modern civilization, cardiovascular diseases (13), diabetes, anti-obesity, anti-inflammatory, antibacterial and antitumor diseases (14), as well as the fight against hepatotoxicity. According to Caper's phytochemical studies, the plant's biologically active substances are physiologically active and widely used in the food industry to protect human health (15).

Therefore, today, Caper species are widely cultivated for their medicinal properties and as a food source. Medicinal species *Capparis spinosa* L. Belongs to the *Capparidace* the most popular species are *C. spinosa* (16*), C. decidua* and *C. ovata* (17*),* the less known are *C. sepiaria, C. tomentosa* and *C. shumilis* (18).

Caper (*Capparis spinosa L.*) grows mainly in the wild, arid regions of Asia, Africa, Saudi Arabia, and Europe, as well as in the Mediterranean basin (19-22).

Cornflower flowers and fruits are consumable materials in the pharmaceutical and food industries. At the same time, its flowers are used in medicine in Asian countries (23, 24).

The average annual world production of Caper is estimated at 10,000 tons, and Spain is one of the leading producers in Europe, with a planted area of about 2,600 hectares and an annual production of 500-1,000 tons (25).

In recent years, plantations of the Caper plant in Uzbekistan have been planted Caper on 3000 hectares by Baraka Meva Industrial Service LLC in the Chust district of the Namangan region and by Zomin Sakhavat LLC in the Zomin district of the Jizzakh region (fig 2) with a volume of 300 ton, started exporting (26).

Various scientific teams in our republic are researching Caper's phytochemical composition and the effect of bioactive substances on the human body (27, 28). It has been established that Caper berries contain many biologically active compounds, such as alkaloids, flavonoids, steroids, terpenoids and tocopherols (29, 30).

However, there is not enough information about the effect of bioactive substances on the body of various organs of the Caper plant (31, 32). Based on the above analysis, the purpose of this research work is to study the immunomodulatory properties of the Caper plant. The immunomodulatory properties of the RAW264.7 line can be conveniently studied in laboratory mice of the macrophage-monocyte line.

Materials and Methods

Samples taken from various organs of the study object *Caper (Capparis spinosa* L.*)* were dried in a room for three days at 21°C. The dried samples were passed through a laboratory mill and turned into powder (Fig.3).

15 g of dried samples were weighed on an electronic balance and extracted with 200 ml of 96% alcohol. The extraction was prepared with constant stirring in an orbital shaker-incubator model ER-20/60 from Biosan at a temperature of 60°C for 24 hours. The remaining 8 ml of prepared samples were removed; after readiness, the samples were cooled, passed through filter paper, and then centrifuged at 3000 rpm for 10 min. Monocyte-macrophage lines cultured from RAW 264.7 macrophage lines of laboratory mice were chosen as the object of study. RAW 264.7 macrophage-monocyte lineage cells were seeded in special plates (6x4=24 wells) the day before exposure to plant extracts. In this case, RAW 264.7 cell cultures were isolated from platelets. Then, it was washed two times with PBS phosphate buffers. To move the cells into the adhesion state, 2 ml of trypsin was added

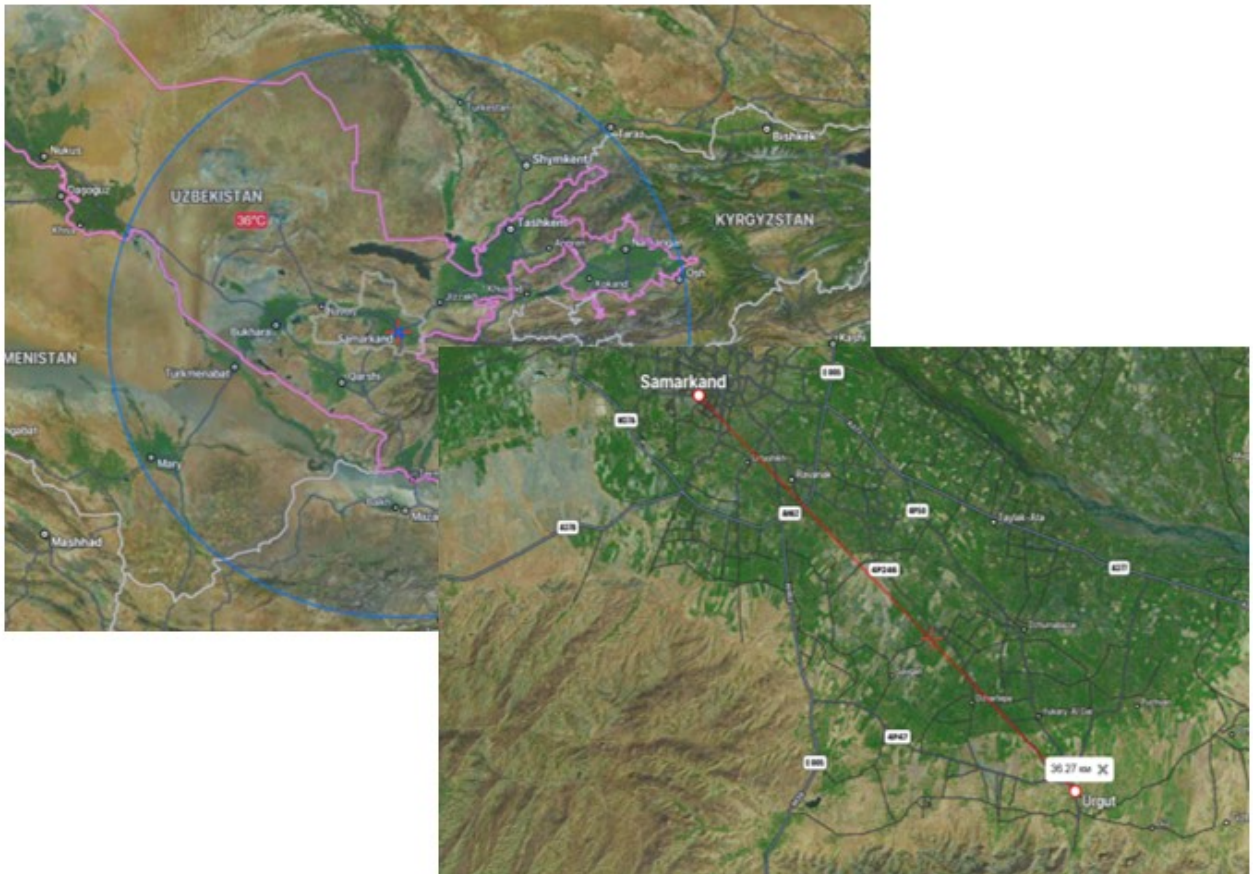

Fig.2. Map of the study area. Urgut mountain range, Samarkand region *(nationsonline.org))*

Fig. 3 Researched parts of *Capparis spinosa L.*

and lightly shaken, then kept in a CO_2 incubator for mouse cells RAW 264.7 is the optimal temperature at 36.6 °C and a humidity of 41% for 5 minutes. Cell cultures adhering to the bottom of the incubated plates were scraped off using a unique tool- a cell scraper. The attached cell cultures were collected and placed in a 5 ml tube. Before transferring cell cultures to new dishes, one drop was dropped into a Thoma chamber (Tiefe Depth Profounder) and counted under a microscope.

5 ml RAW 264.7 cell lines were mixed with 50 ml DMEM using a pipette, and 0.5 ml RAW 264.7 cells were seeded into two plates with 48 wells. These cells were stored in a CO_2 incubator at 36.5°C and a humidity of 41% for one day. Then, in two treatments, they were given a control: 25, 5, 1 µl per first tablet and 1, 1, 0.5, 2 µl per second tablet with different concentrations of flowers, fruits, seeds, roots, leaves, stem extracts and inflammation Caper. Bacteria were exposed to lipopolysaccharide-LPS in 0.5 µl to provide a therapeutic effect.

Statistical analysis of data obtained from experiments was performed using GraphPad Prism version 10. Results were expressed as mean ± SEM. One-way and two-way ANOVA followed by Bonferroni and Tukey post hoc multiple comparisons, respectively, to assess statistical significance in different models, and a P value of <0.05 was considered significant.

Results and Discussion

It is known that many researchers have been studying articles on the medicinal properties of Caper (*Capparis spinosa* L.). The results of studies of the medicinal properties of the stem, leaf, flower, fruit, seeds and roots of various organs of Caper by many authors differ from each other, and the primary research consists in determining the chemical composition of different organs of Caper (*Capparis spinosa* L.). It is devoted to determining the medicinal properties.

Also, several researchers in Uzbekistan have covered the medicinal properties of the Caper (*Capparis spinosa* L.) plant, its chemical composition and trace elements, and its agrotechnical properties. The Caper (*Capparis spinosa* L.) extract is predominantly regenerative, and immunomodulatory medicinal properties have been identified in treating haemorrhoids. Among the chemical substances in its composition, mainly capparin A, B, C, flazin, guanosine, 1-H-indole-3-carboxaldehyde, apigenin, kaempferol, tevetiaflavone, rutin, quercetin, glucocaparin, stachidrine were identified and determined using spectrophotometric and chromatographic methods.

Based on these data, the influence of the immunomodulatory properties of the plant Caper (*Capparis spinosa* L.) on mouse macrophage cells RAW 264.7 was studied in morphological tests.

In the studies, 5 ml of RAW 264.7 cell line was mixed with 50 ml of DMEM using a pipette and 0.5 ml of RAW 264.7 cells were seeded into two plates with 48 wells. These cells were stored in a CO_2 incubator at 36.5°C and a humidity of 41% for one day. Then, in two different treatments, they were given a control: 25, 5, 1 µl (Fig. 4) and 1, 1, 0.5, 2 µl (Fig. 5) with different concentrations of flowers, fruits, seeds, roots, leaves, stem extracts and inflammation Caper. Bacteria were exposed to lipopolysaccharide-LPS in 0.5 µl to provide a therapeutic effect. The study design is shown in Table 1 and Fig. 4 and 5.

The following control samples were prepared. 0.5 µl of RAW 264.7 cells were exposed to 0.5 µl of LPS. In the second control, 0.5 µl of RAW 264.7 cells were used as a control. The third option was to expose 0.5 µL of RAW 264.7 to 1 µL of 96% alcohol. In the fourth variant, 25 µl of 96% alcohol was added to 0.5 µl of RAW 264.7 cells.

In the control variant, when RAW 264.7 cells were exposed to 0.5 µl of LPS, macrophage cells had high proliferative activity; in III of the control variants, 1 µl of a

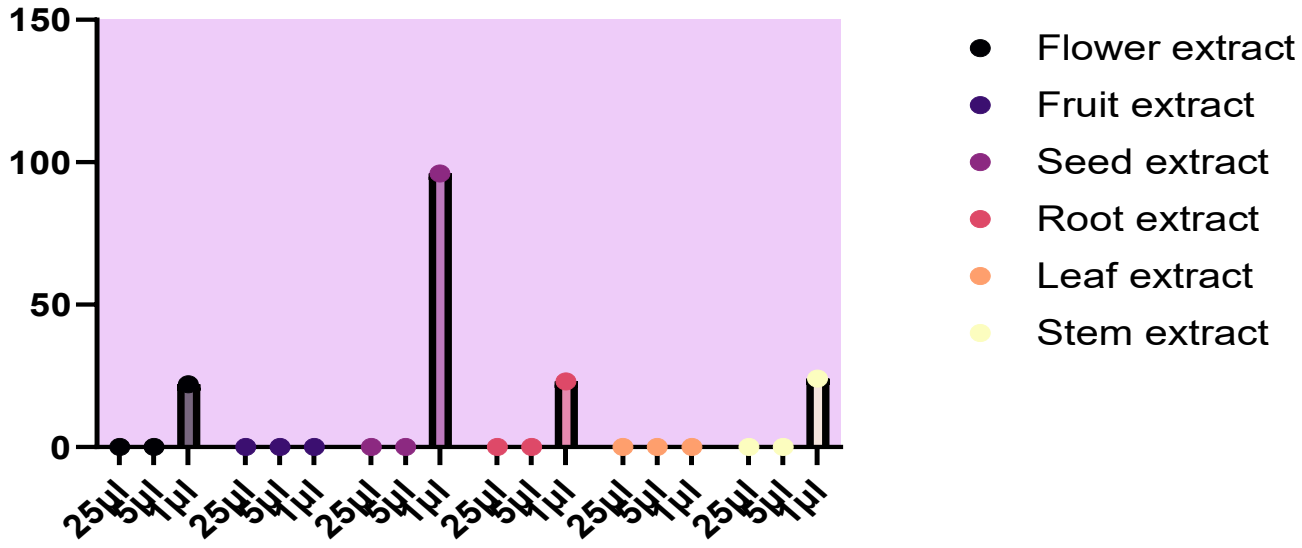

Fig. 4 Application of Caper *(Capparis spinosa* L.*)* in different (2,0,5 1 µl) concentrations

Table 1. Level of proliferation at different concentrations of *Capparis spinosa L.*

Sample Rate	quantity	RAW 264.7 (0.5 µl) + LPS (0.5 µl)	quantity	RAW 264.7 (0.5 µl) + LPS(0.5 µl)
	control			
	1 µl	-	1 µl	+
	25 µl	-	1 µl	++
Flowers extract	5 µl	-	0,5 µl	+++
	1 µl	+	2 µl	++
	control			
	1 µl	-	1 µl	+
Fruit extract	25 µl	-	1 µl	++
	5 µl	-	0,5 µl	+++
	1 µl	+	2 µl	++
	96% alcohol			
	1µl control	-	1 µl	-
	25 µl	-	1 µl	+
Seed extract	5 µl	-	0,5 µl	+
	1 µl	+++	2 µl	-
	96% alcohol			
	1µl control	-	1 µl	-
	25 µl	-	1 µl	++
Root extract	5 µl	-	0,5 µl	+++
	1 µl	+	2 µl	+
	96% alcohol			
	1µl control	-	1 µl	-
	25 µl	-	1 µl	-
Leaf extract	5 µl	-	0,5 µl	-
	1 µl	-	2 µl	-
	96% alcohol			
	1µl control	-	1 µl	+
	25 µl		1 µl	++
	5 µl	-	0,5 µl	++
Stem extract	1 µl	+	2 µl	+

Note:-no proliferation, +- not high proliferation, ++-moderate proliferation, +++- high proliferation.

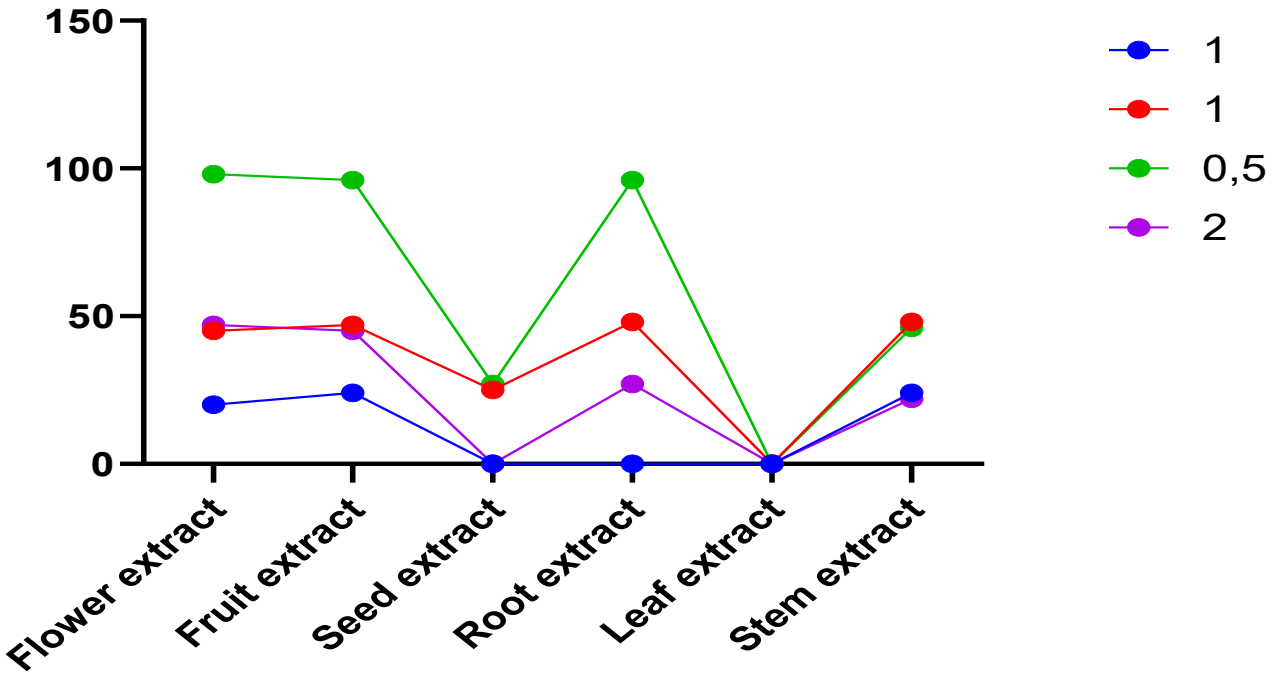

Fig. 5. Application of Caper (*Capparis spinosa* L.) in different (2,0,5 1 µl) concentrations.

96% alcohol solution was added to 0.5 µl of RAW 264.7 macrophages, LPS was not added. Samples of 0.5 µl of RAW-264.7 macrophages died under the influence of 25 µl of 96% alcohol.

Variants were created based on the results obtained, and the first plate was treated with 25 µl, five µl and one µl of Caper (*Capparis spinosa* L.) flower extract per 0.5 µl RAW 264.7, 0.5 µl LPS according to the information given in figure 4.

When the proliferation of RAW 264.7 macrophage cells was examined under the microscope field under the influence of 25 µl of flower extract, it was found that all cells died (Fig. 6).

Further, when 0.5 µL of RAW-264.7 was exposed to 0.5 µl of LPS followed by one µl of flower extract and incubated for one day in a CO_2 incubator, the incubation results were morphologically examined under a microscope, macrophage cells did not have high proliferative activity, the cells did not die (Fig. 7), under the influence of Caper 25 fruit extract, five µl, no proliferation of RAW 264.7 macrophage cells was observed in the field of view of the microscope, and the cells died (Figure 6).

Fig. 6. Control variant - RAW 264.7 cells stimulated with LPS.

After stimulation of 0.5 µl of RAW-264.7 with 0.5 µl of LPS and after incubation with exposure to 1 µl of fruit extract, during a morphological study of the results of incubation under a microscope, RAW 264.7 macrophages in 1 µl of fruit extract, low proliferative activity was observed in the cells (Fig. 7).

Extracts of seeds of (*Capparris spinosa* L.) in concentrations of 25 and 5 µl did not cause the proliferation of RAW 264.7 cells; the cells died (Fig. 6).

0.5 µl of RAW-264.7 was exposed to 0.5 µl of LPS, followed by one µL of seed extract and stored in a Caper *(Capparis spinosa* L.*)* incubator for one day. After 16 hours of incubation, the results were studied morphologically under a microscope. The highest proliferation activity was observed in RAW 264.7 macrophage cells in 1 µl of seed extract (Fig. 7). Under the influence of Caper *(Capparis spinosa* L.*)* root extract 25.5 µl, no cell proliferation was observed in the field of view of the microscope - macrophages RAW 264.7, and the cells died (figure6).

To the information presented in Fig. 4. 1, 25 µl, 5 µl and 1 µl of Caper (*Capparis spinosa* L.) leaf extract were obtained. The results of the study, under the influence of Caper (*Capparis spinosa* L.) leaf extract 25, 5, 1 µl, no increase in the proliferation of RAW macrophage cells was observed 264.7 in the field of view of the microscope, the cells died (Fig. 8). Under the influence of Caper (*Capparis spinosa* L.) stem extract, 25, 5 µl, in the field from the microscope, no proliferation of RAW 264.7 macrophage cells were observed, and the cells died (Fig. 6).

In the 2nd stage of the study, the concentration of the samples was changed. Based on the results obtained, samples of flower extracts were prepared. The data presented in Fig. 5, RAW 264.7 was formed in the amount of 0.5 µl, 0.5 µl LPS, 1 µl, 0.5 µl and 2 µl of Caper (*Capparis spinosa* L.) flower extract.

The control variant was added to the variants available for the study. 0.5 µl of RAW 264.7 cells in each well of the plate was exposed to 1 µl of individual extracts of flowers, fruits, seeds, roots, leaves and stems.

In the control treatment, when 0.5 µl of RAW 264.7 cells were exposed to 1 µl of flower extract, the

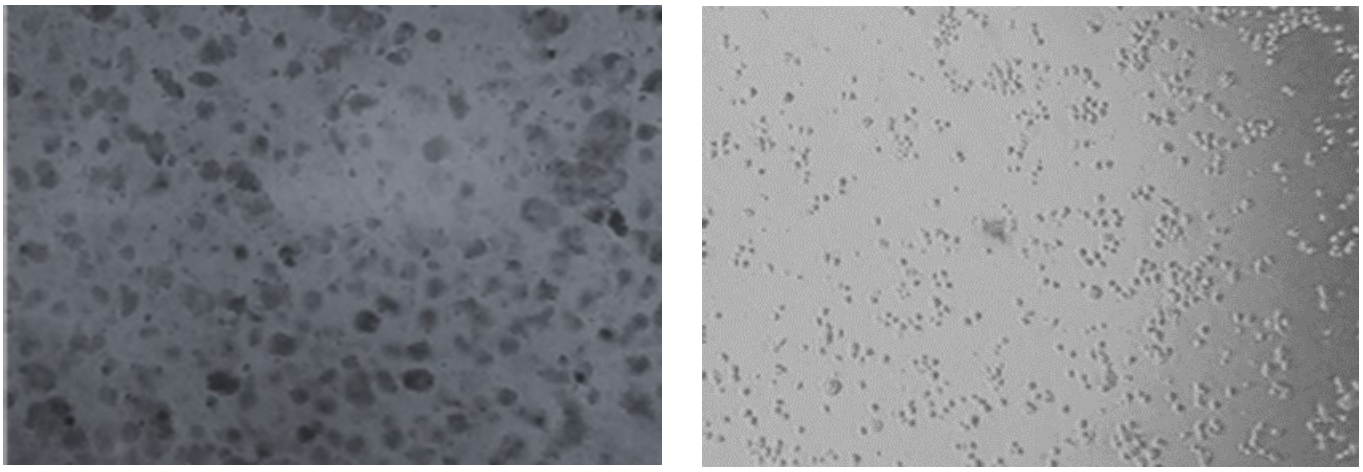

Fig. 7. Morphological appearance of cells in 1 µl of different extracts of *Capparis spinosa***.**

proliferation activity of macrophage cells was observed. As a control, when 0.5 µL of RAW 264.7 cells were exposed to 1 µL of fruit extract, macrophage cells did not exhibit high cell proliferation activity when examined under the microscope field, 1 µl of seed extract, macrophage cells died, and 1 µl of root extract, macrophage cells did not actively proliferate.

0.5 µL of RAW 264.7 cells were exposed to 1 µL of leaf extract; no proliferation activity was observed in the macrophages and the macrophages were killed (Fig. 8); 1 µl of stem extract and macrophage proliferation was observed.

Figure 5 shows RAW 264.7 was formed in the amount of 0.5 µl, 0.5 µl LPS, 1 µl, 0.5 µl and 2 µl of Caper (*Capparis spinosa* L.) flower extract. Under the influence of 1 µl of extract flowers of Caper (*Capparis spinosa* L.), an increase in the proliferation of RAW 264.7 macrophage cells in the microscope's field of view was established.

In 1 µl of flower extract, the number of RAW 264.7 cells did not increase significantly, but the sizes of macrophages and monocytes increased, and in 0.5 µl of flower extract, high proliferative activity of RAW 264.7 cells were observed rice, in 2 µl of flower extract, the number of RAW 264.7 cells increased slightly, but the size of macrophages and monocytes increased. According to the results of studies under the influence of Caper (*Capparis spinosa* L.) fruit extract spinosa L. 0.5 µl in the microscope's field of view, an increase in the proliferation of RAW 264.7 macrophage cells was observed.

The number of RAW 264.7 cells increased significantly in 0.5 µl of fruit extract (Figure 9), while the number of RAW 264.7 cells increased substantially in µl of fruit extract, the number of RAW 264.7 cells increased significantly in 2 µl of fruit extract.

Based on the results obtained, samples of seed extracts were prepared. According to the data presented in Table 1, RAW 264.7 was treated with 0.5 µl, 0.5 µl LPS, 1 µl, 0.5 µl and 2 µl Caper (*Capparis spinosa* L.) seed extract. According to the study's results, under the influence of Caper (*Capparis spinosa* L.) seed extract 0, 5 µl in the microscope's field of view, an increase in the proliferation of RAW 264.7 macrophage cells was observed.

In 0.5 µl of seed extract, RAW 264.7 cells had high proliferative activity. In 1 µl of seed extract, the number of RAW 264.7 cells increased relatively. In 2 µl of seed extract, RAW 264.7 cells died.

According to the study's results, under the influence of 0.5 µl of root extract, the proliferation of RAW 264.7 macrophage cells was observed in the microscope's field of view.

High proliferative activity was observed in RAW 264.7 macrophage cells in 1 and 0.5 µl of root extract, microscopic data confirm an increase in the volume of

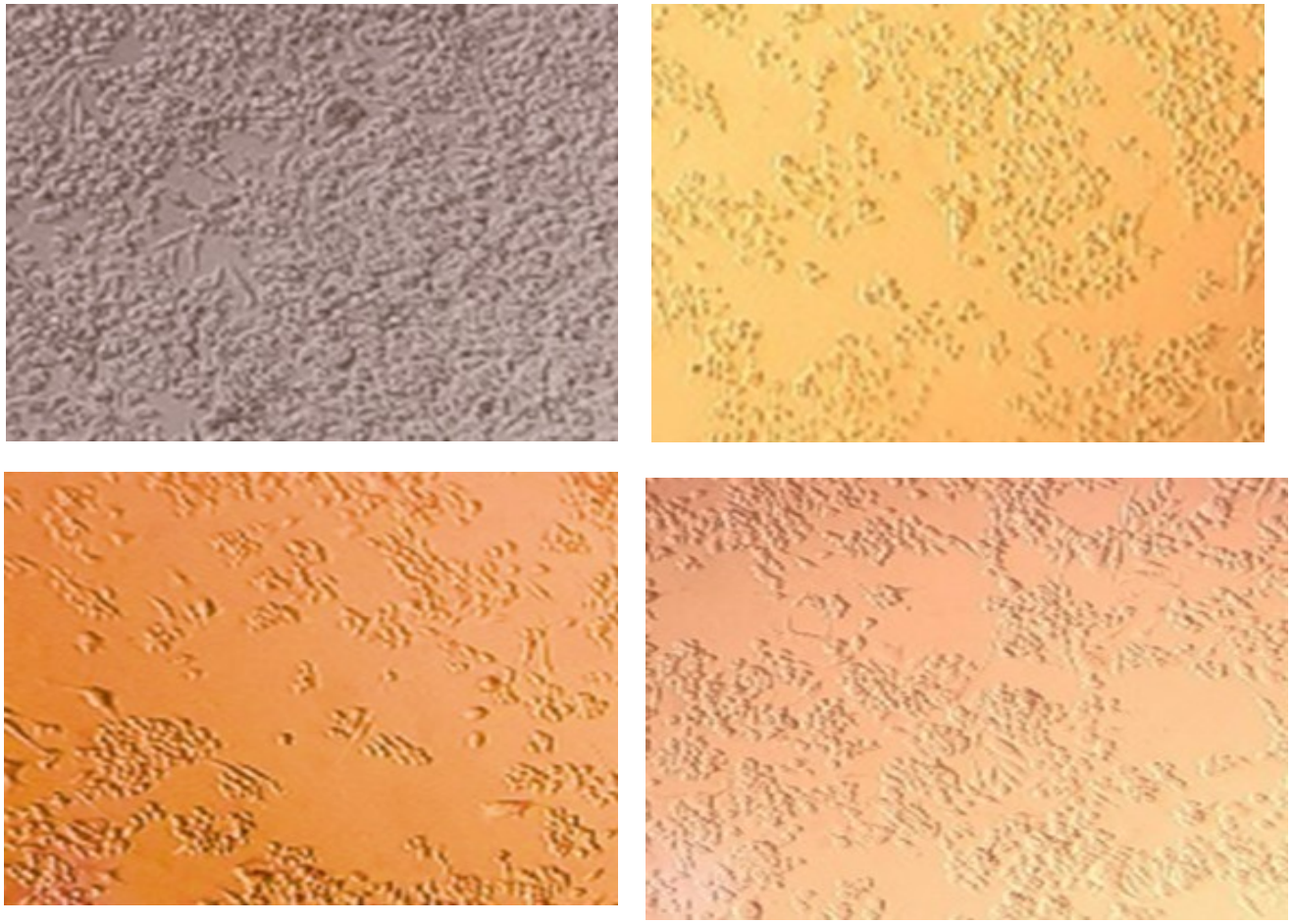

Fig. 8 Cells died in 25, 5 and 1 µl of leaf extract.

macrophages.

In 2 µl of root extract, low proliferative activity was observed in RAW 264.7 macrophage cells. According to the study results, under the influence of Caper (*Capparis spinosa L.*) leaf extract L. 0.5 µl, no proliferation of RAW 264.7 macrophage cells was observed in the microscope's field of view. Proliferation of RAW 264.7 macrophage cells was not observed under the influence of 0.5 µl of LPS 1, 0.5, 2 µl of root extracts to 0.5 µl of RAW-264.7 in the microscope's field of view, the cells died.

The Caper (*Capparis spinosa* L.) stem extract, in a volume of 0.5 µl, increased the proliferation of RAW 264.7 macrophage cells, which was observed in the microscope's field of view.

After exposure of rat RAW-264.7 cells to 0.5 µl of LPS and 1 µl of stem extract, the incubation results were examined morphologically under a microscope. In 1 µl of stem extract, the number of RAW 264.7 cells increased significantly; in 0.5 µl of stem extract, no proliferative activity was observed in RAW 264.7 macrophage cells; in 2 µl of stem extract, moderate proliferative activity was observed in RAW 264.7 macrophage cells.

Conclusion

As a result of the experiments, it was found that when monocyte-macrophage mouse cells were exposed to RAW 264.7 plant extracts at a concentration of 25 and 5 µl, the cells in all samples died. At a concentration of 1 µl, it was observed that the proliferative activity of cells is low.

However, the proliferation level had high indicators due to the effect of 1 µl seed extract (*Capparis spinosa* L.). In a control variant, mouse RAW 264.7 cells were stimulated with LPS.

In the later stages of the study, proliferation rates were not high in cells that were not stimulated with LPS. In the control variant, cells were destroyed with 1 µl of leaf and root extract. In cells stimulated with LPS, the level of proliferation was high at a concentration of 0.5 µl of flower, fruit and root extracts. An average level of proliferation was observed in 1 µl of extracts of flowers, fruits and roots, as well as in 1 and 0.5 µl of stem extracts. At a concentration of 2 µl of plant parts, the level of cell proliferation was low. Seed extract concentrations of 1 and 0.5 µl had low proliferation rates, and cells died at a concentration of 2 µl.

Caper (*Capparis spinosa* L.) cytotoxicity was observed in leaves with cell death at all concentrations of leaf extract. The highest level of plant proliferative activity was observed at 0.5 µl of flower extract and 0.5 µl of fruit and root extract. The level of cell proliferation was observed at a concentration of 1 µl of seed extract.

High proliferative activity is possibly due to the interaction of chemical components promoting mitotic activity.

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Authors' contributions

S.X., N.F., G.D., S.K. and G.A. performed the experiments. S.X., N.F., and G.D. analyzed data and statistically analyzed results. All authors wrote the draft of the manuscript. S.K. and G.A. conducted the critical revision of the manuscript. S.X. and G.D. worked out the concept and design supervised and funded the experiments. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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