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ORIGINAL ARTICLE

The Physiological and Genotoxic Effects of Water and Ethanol Extracts of Goji Berry (*Lycium barbarum* L.) on Model Organisms

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ABSTRACT

Objectives: Herbal treatments in particular have a place which cannot be ignored in current alternative medicine. One of these plants, which has become very well-known recently and which is widely consumed in Turkey is the Goji berry (*Lycium barbarum* L.). To investigate the effects of Goji berry, on fertility when used by individuals in their 20s and on physical growth when used by those of a younger age. It was also aimed to test the genotoxic effects of the Goji berry on *Allium cepa* L. meristem stem cells.

Materials and Methods: Goji berry water and ethanol extracts were tested on a model organism of *Caenorhabditis elegans* (*C. elegans*) in respect of fertility and physical growth. *Allium cepa* L. meristem stem cells exposed to Goji berry extract were examined for genotoxic effects.

Results: A significant dose-related reduction was determined in fertility, physical growth and mitotic index in ethanol extract. In the water extract, a reduction in fertility was determined at high doses and an increase with the lowest dose used in the study. No significant effect was found on physical growth of water extract at any dose.

Conclusion: The results of this study showed negative effects on cell division, fertility and physical growth of Goji berry, which has been used since ancient times in Chinese medicine and for which several benefits have been claimed in literature. It must be emphasised that dose-related side-effects may develop with the use of plants for medicinal puposes.

Key Words: Goji Berry, *Caenorhabditis elegans*, Fertility, Physical growth, Genotoxic effects

INTRODUCTION

Throughout human history, according to knowledge gained by our ancestors, several methods have been used to benefit from natural resources and particularly of plants in the treatment of diseases. Despite the developments in medicine in recent years, this tradition has continued, especially in developing societies, due to both ease of availability and for economic reasons [1- 4]. Traces of the human use of plants for different puposes such as a food source and treatment have been found in fossils 60,000 years old from the Mid-Paleolithic Age. Morphine, which was isolated in 1803-1804 by Derosne and Seguin and in 1805 by Frederich Serturner and digitalis which was obtained by Nativelle in 1868 were the first plant source components used as medicinal effect substances [5].

Herbal treatments in particular have a place in current alternative medicine of a dimension which cannot be ignored. Following some processing, several plants are used for the treatment of various diseases, to improve physical appearance or for cosmetic purposes. One of these which has been much heard of recently is the Goji berry (*L. barbarum* L), which is of Asian origin. The Goji berry is a plant of the Solanacae family. The red-coloured fruits have been used for treatment purposes for thousands of years in Chinese medicine. The known effects include anti-ageing, regulation of kidney and

liver functions, neuroprotective effects, relieving fatigue, accelerating metabolism, diabetic glucose control, anti-glaucoma effects, anti-oxidant effects, immunomodulation, anti-tumour effects and cytoprotection. Studies have shown that these effects originate from the polysaccharides found in the Goji berry [1, 2, 4, 6].

As *C. elegans* is similar to the human genome, they have been widely used in many recent scientific studies as a model organism [3, 7-9]. It is a soil-dwelling, apathogenic nematode, 1mm in length, with two genders of male and hermaphrodite. The hermaphrodite produces sperm and eggs and impregnates itself. A mature hermaphrodite can leave approximately 300 eggs in a lifetime. It was the first organism in which the genome series was determined. There are approximately 20,000 genes and a total of 6 chromosomes. It has been determined that a significant proportion of these genes show a great similarity to human genes [10].

There are few studies in literature on the effect of Goji berry on fertility and growth. In the current study, while the effect on fertility and growth was tested on a model organism of *C. elegans,* the genotoxic effects were examined on *Allium cepa* L meristem stem cells exposed to Goji berry. *A. cepa* L is a frequently used control test in the investigation of cytotoxic effects [11]. That abnormalities in mitotic cells and the mitotic index can be easily determined are advantages of this method.

The use of Goji berry, which is also known as wolfberry, is extremely widespread in Turkey for weight-loss purposes, especially by those in their 20s and also for anti-diabetic purposes because of the glucose regulating effect. The aim of this study was to investigate the effects of Goji berry, on fertility when used by individuals in their 20s and on physical growth when used by those of a younger age. It was also aimed to test the genotoxic effects of the Goji berry on *A. cepa* L. meristem stem cells.

MATERIALS AND METHODS

Obtaining the Goji berry (*L. barbarum* L.) and preparation of the water and ethanol extracts

The Goji berry was purchased in dried form of approximately 100 gr from 5 different shops in Sivas, Turkey. The total 500 gr of fruit were crushed in a homogenisator then 500 mL deionised pure water was added. The mixture with the water was processed for 48 hours on an agitator, then filtered through a Whatman No 1 filter. With an evaporator, the extract solvent was evaporated then the extraction process was repeated with deionised pure water. Ethanol extract was obtained by conducting the same extraction method with ethanol.

The effect of Goji berry (*Lycium barbarum* L.) water and ethanol extract on fertility and physical growth in *C. elegans*

The water and ethanol extracts of Goji berry were investigated separately. The effects of Goji berry water and ethanol extracts were examined on a model organism with *C. elegans*. The N2 wild type *C. elegans* were used and these strains were obtained from Minnesota University Caenorhabditis Genetics Center.

Synchronisation of *C.elegans*

Approximately 20 mature *C. elegans* were transferred to a petri dish of Nematode Growth Media (NGM) containing *E.coli.* After the production of eggs in a period of 4-6 hours, the mature *C. elegans* were removed from the petri dish. These eggs produced synchronised offspring. After 3 days when these reached adult form, they were used in the study. To be able to provide sufficient *C. elegans* for the study, 5 petri dishes were used simultaneously.

Nematode growth media (NGM)

2.5 gr Peptone, 3 gr NaCl, and 20 gr Agar were dissolved in 1 L of distilled water and after 15 mins autoclave at 125°C, the solution was cooled to 55°C. Then a previously prepared buffer of 1mL $MgSO_4$ (1M), 1 mL Cholesterol (5 mg/mL), 1 mL $CaCl_2$ (1M), 25 mL KH_2PO_4 (pH:6) which had been filtered through a 0.2 µm mesh was added to the medium and homogenisation was obtained. The water and ethanol extracts of Goji berry at defined doses were added separately to 100 mL NGM (0.5 gr / 100 mL, 0.25 gr / 100 mL, 0.125 gr / 100 mL, 0.06 gr / 100 mL). From the prepared NGMs, amounts of 10 ml were transferred to 6cm petri dishes and after thickening, inactivated *Escherichia coli* OP50 strains which had been produced in Lauryl Sulfate Broth (LSB) and kept at 60 °C for 30 mins were added to the NGM and dried in a sterile cabinet. For the control group, no extract was added to the prepared NGM.

Ferility analysis

The egg count used in the fertility analysis was applied according to the Koelle [12] protocol. To each separately prepared petri dish, 25 well-nourished L4 form *C. elegans* were transferred with the Goji berry water and ethanol extract. After 24 hours, 20 of these were transferred to a new petri dish and kept at 20°C for 60 mins. At the end of this period, the eggs were counted at x20 magnification and were compared with the control group.

Physical growth evaluation

For the evaluation of physical growth, an equal number of N2 wild type *C. elegans* eggs were added to the NGM containing Goji berry extracts. The petri dishes, each containing 90-100 eggs were examined daily in respect of offspring produced from the eggs and physical growth and comparison was made with the control group.

The studies were conducted at 20°C and 5 petri dishes were used for each dose. Each study was repeated twice.

The genotoxic effect of Goji berry water and ethanol extract on *A. cepa* L. meristem stem cells.

The *A. cepa* L. used as the study material were left to germinate for 2 days in pure water at room temperature. The germinated

A. cepa L. were exposed for 12 and 24 hours to defined dilutions of Goji berry water and ethanol extracts (0.5 gr / 100 mL, 0.25 gr / 100 mL, 0.125 gr / 100 mL, 0.06 gr / 100 mL). No extract was added to the control group. At the end of this period, the *A. cepa* L. were cut with scissors close to the root tips and were fixed in ethyl alcohol-glacial acetic acid (3:1) fixative for 24 hours at +4 °C. The fixed stems were kept in 1N HCl solvent for 10 mins. Slides were prepared with the crush method from the stem tips stained with aceto orcein stain. At least 2000 cells for each dose and control were examined under a microscope in respect of genotoxicity. The findings were compared with the control group.

Statistical analysis

Statistical analyses were made using SPSS v. 21.0 software. One-way ANOVA variance analysis was applied to the data obtained in the study. Differences between the groups of a significant level were determined with the Tukey Multiple Comparison Test. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

The defined doses of Goji berry water and ethanol extracts were examined separately in the study. First the fruit extracts were tested on the fertility of a model organism of *C. elegans*. The results of the study showed that there were negative effects on fertility of both extracts at the dose of 0.5 gr/100 mL and the number of eggs was determined to have statistically significantly reduced ($p<0.05$). At the doses of 0.25 gr/100 mL, 0.125 gr/100 mL and 0.5 gr/100 mL, the egg count was reduced and although the difference was not very great in the water extract, the difference was found to be statistically significant ($p<0.05$). At a dose of 0.06 gr/100mL of water extract, the egg count was determined to have increased compared to the control group ($p<0.05$).

At all the doses of ethanol extract, the egg count of the model organism was determined to be statistically significantly reduced ($p<0.05$, Table 1).

Table 1. The effect of Goji berry (*L. barbarum* L.) water and ethanol extract on the fertility of *C. elegans*

Doses (g /100mL)	Ethanol extract (Mean / Number)	Water extract (Mean / Number)
0.5	19*	39*
0.25	34*	256*
0.125	36*	263*
0.06	49*	291*
Control	286	286

* The difference in test groupswas significant compared to the control group ($p<0.05$). One-Way Anova Test

At the second stage of the study, the newly-hatched *C. elegans* L1 forms were exposed to defined doses of Goji berry water and ethanol extracts to investigate the effects on growth. No statistically significant effects of water extracts were determined on growth ($p>0.05$). Ethanol extracts at doses of 0.5 gr/100 mL, 0.25 gr/100mL and 0.125 gr/100 mL were determined to have caused almost no dose-related growth in the model organism (Table 2, Figure 1). This finding was statistically significant.

Table 2. The effect of Goji berry (*Lycium barbarum* L.) water and ethanol extract on the physical growth of *C. Elegans*

Doses (g /100mL)	Ethanol extract (Mean/mm)	Water extract (Mean/mm)
0.5	0.6*	2.0
0.25	0.9*	2.0
0.125	1.2*	2.1
0.06	2.0	2.2
Control	2.3	2.3

*The difference compared to the control group was significant ($p<0.05$). One-Way Anova Test, Measurements were made at x 2 magnification

At the third stage of the study, the genotoxic effects of Goji berry water and ethanol extracts were examined on *A. cepa* L. meristem stem cells (Tables 3 and 4, Figure 2). The germinated *A. cepa* L. meristem stem cells were exposed to defined doses of Goji berry water and ethanol extracts. At two time periods of 12 and 24 hours, more than 2000 cells were examined for each dose. In the cells exposed to ethanol extract, cell division was determined to have been prevented to a significant degree at almost all the doses and the mitotic index was decreased (Table 3). At the end of 12 hours, of the 2208 cells examined in the control group, prophase was seen in 23.64 %, metaphase in 5.16% and anaphase/telophase in 2.85%. In the 2136 cells examined at the dose of 0.5 gr/100 mL, prophase was observed in 1.68%, metaphase in 0.51% and anaphase/telophase in 0.14%.

At the end of the 24-hour period, 2153 cells were examined in the control group. Of these, prophase was seen in 25.82 %, metaphase in 5.48 % and anaphase/telophase in 2.69 %. In the 2126 cells examined at the dose of 0.5 gr/100 mL ethanol extract at the end of 24 hours, prophase was observed in 1.36 %, metaphase in 0.42 % and anaphase/telophase in 0.23 %.

In the 12-hour period the mitotic index was decreased by 31.7% in the control group, and by 2.3% at the 0.5gr/100mL dose of ethanol extract. These rates were 34.0% and 2.02% at the end of 24 hours. Similar reductions were determined at the other doses. A statistically significant decrease was determined in the mitotic index at all doses of ethanol extract compared to the control group ($p<0.05$).

As a result of the examination of genotoxicity in the *A. cepa* L. meristem stem cells exposed to water extract, the mitotic index was observed to have decreased but not at the same rate as for ethanol extract. At the end of 12 hours, this rate was determined to have receded to 31.7% in the control group and to 26.5% in

the 0.5gr/100mL dose group. This rate was 27.5% at the end of 24 hours ($p<0.05$). At the other doses, a similar dose-related reduction was determined ($p<0.05$), and the difference between the 0.25gr/100mL and 0.125gr/100mL doses was not determined to be statistically significant ($p>0.05$, Table 4). When the chromosome structure in the cells was examined, while no anomaly was determined in the control group at the end of 12 and 24 hours, in the ethanol extract group, the C-mitosis rate was found to be 6% at 0.5 gr/100mL dose, 4.4% at 0.25 gr/100mL, 1.4% at 0.125 gr/100mL and 0.3% at 0.06 gr/100mL. At the end of 24 hours these rates were 9.3%, 4.8%, 2.16% and 0.52%, respectively.

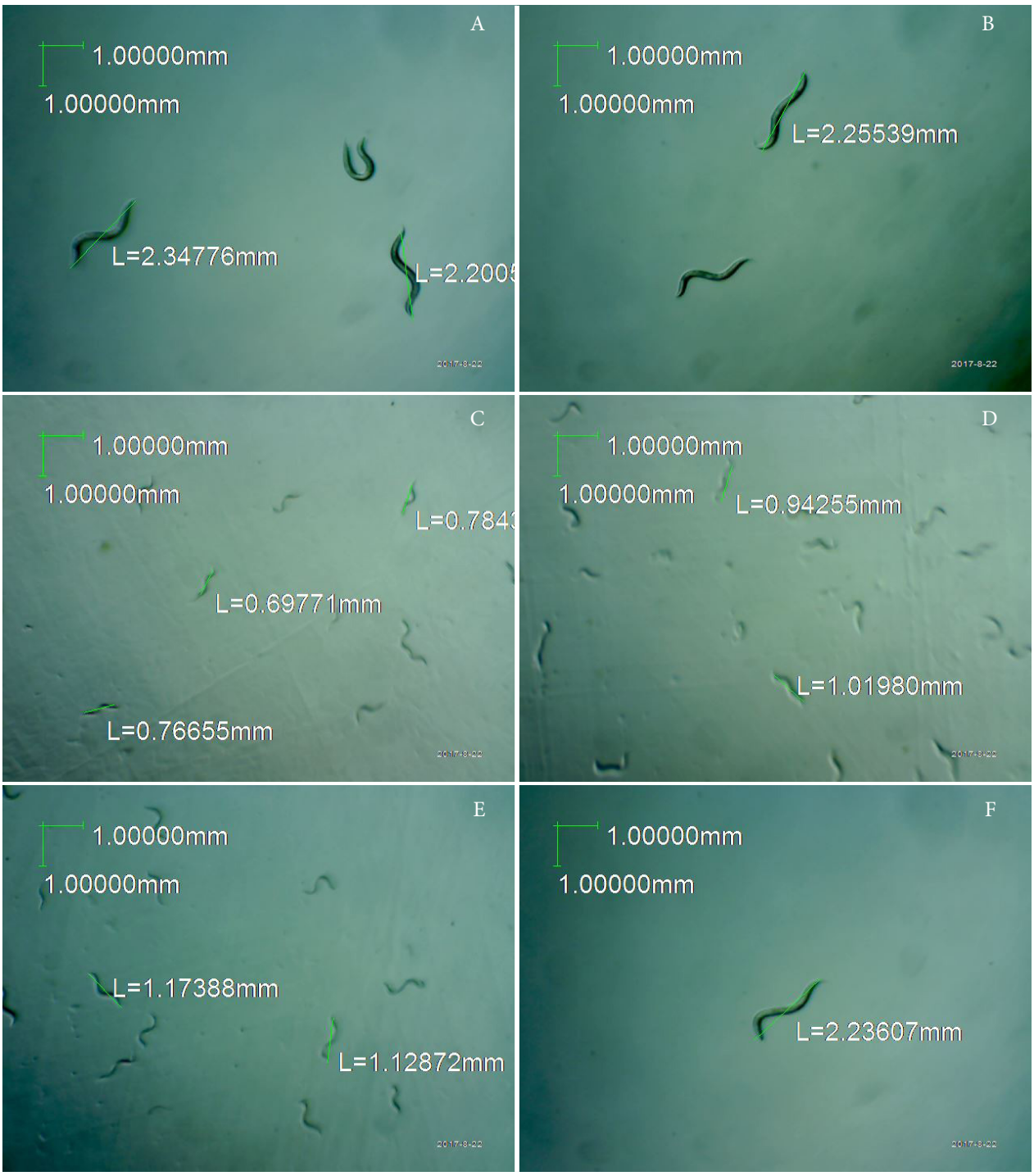

Figure 1. The effect of Goji berry ethanol extract on fertility and physical growth in *C. elegans*, 2x, (A,B; Control, C; Ethanol extract 0.5 gr, D; Ethanol extract 0.25 gr, E; Ethanol extract 0.125 gr, F; Ethanol extract 0.06 gr.)

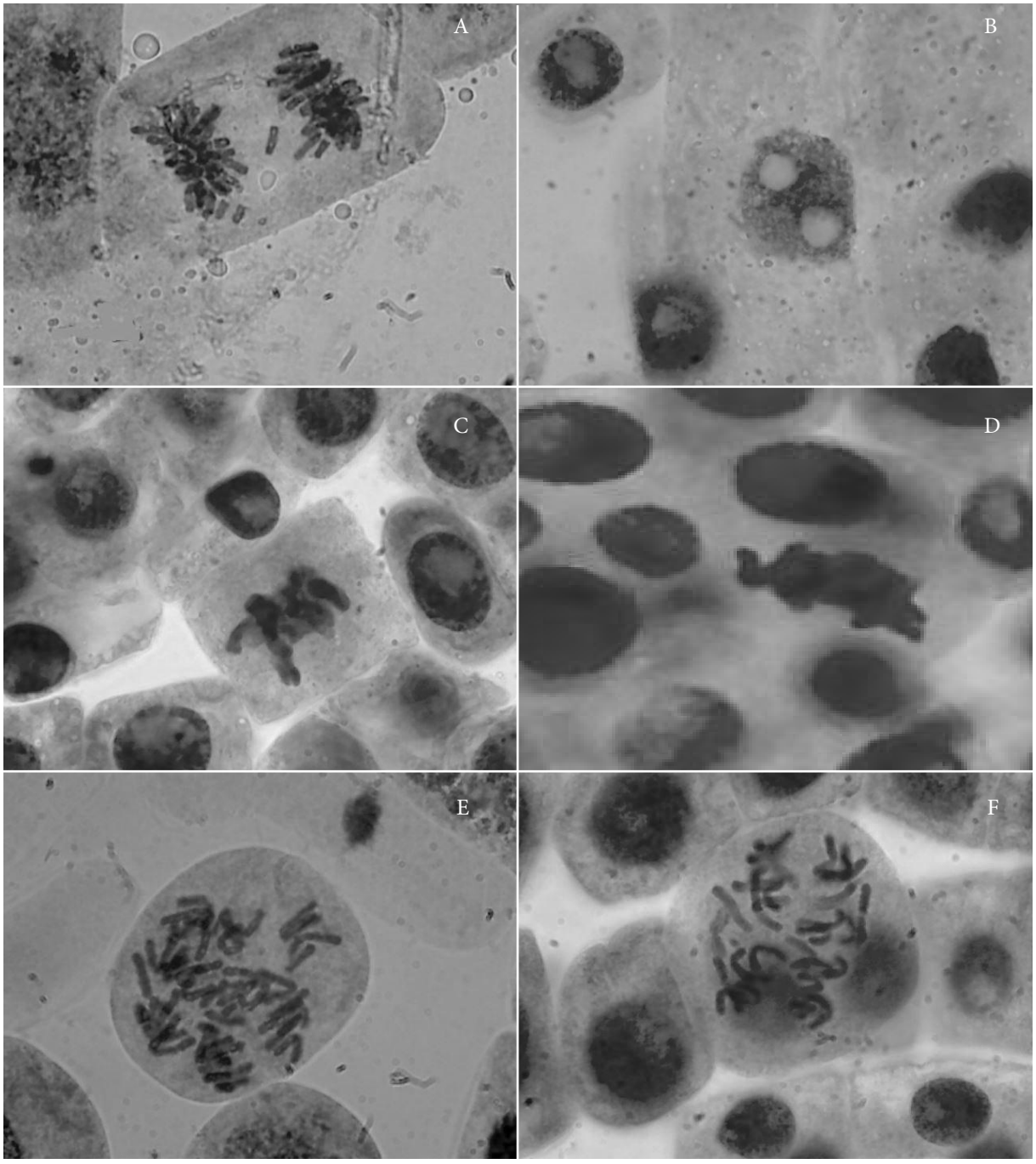

Figure 2. Different types of aberrations induced by ethanol extracts of Goji berry in *Allium cepa* L. root tips, 100x. (A; Laggard chromosome, B; Binucleated cell, C, D; Sticky metaphase, E, F; C-mitosis)

Anaphase bridging was determined in ethanol extract only at the end of 24 hours in the 0.5gr/100mL dose at the rate of 2.4% and the stickiness rate was determined to be dose-related at high rates. In the 12-hour period, these rates were observed to be 8% at 0.5 gr/100mL dose, 3.07% at 0.25 gr/100mL, 1% at 0.125 gr/100mL, and 0.51% at 0.06 gr/100mL. At the end of 24 hours, these rates were determined as 9.3%, 2.19%, 0.65%, and 0.52%, respectively.

Laggard chromosome was determined after 12 hours in the 0.25gr/100mL dose at the rate of 0.38% and after 24 hours at 4.65%. When the chromosome anomalies were examined in the *Allium cepa* L. meristem stem cells exposed to Goji berry water extracts, these rates were seen to be very high. At the end of 12 hours, C-mitosis was determined at 0.34% in 0.5gr/100ml dose and at 0.17% at the end of 24 hours in the same dose. The stickiness rate was determined as 0.51% in 0.5 gr/100mL dose and 0.16%

in 0.25 gr/100mL after 12 hours and as 0.34% in 0.5gr/100mL after 24 hours.

When the rates of total damaged cells were examined from the divided cells, this rate was determined as 0% in the control group, and after 12 hours, 14% at 0.5 gr/100mL ethanol extract dose, 8.07% at 0.25 gr/100mL, 2.42% at 0.125 gr/100mL, and 0.85% at 0.06 gr/100mL. After 24 hours these rates were determined as 25.5% at 0.5 gr/100mL ethanol extract dose, 7.01% at 0.25 gr/100mL, 2.81% at 0.125 gr/100mL, and 0.52% at 0.06 gr/100mL (Tables 3 and 4, Figure 2).

Table 3. Cytogenetic analysis of *A. cepa* L. root tips exposed to different concentrations of Goji berry ethanol extracts for different periods

Time of treatment	Doses (gr / 100 ml)	Examined total cells	Prophase (%)	Metaphase (%)	Anaphase–Telophase (%)	Mitotic index (%)*	C-mitosis (%)	Anaphase bridges (%)	Stickiness (%)	Laggards (%)	Total abnormalities (%)*
12 h	Control	2208	23.64	5.16	2.85	31.7[a]	0	0	0	0	0[a]
	0.5	2136	1.68	0.51	0.14	2.3[b]	6	0	8	0	14[b]
	0.25	2058	10.49	1.36	0.77	12.6[c]	4.6	0	3.07	0.38	8.07[c]
	0.125	2105	18.47	3.04	1.99	23.5[d]	1.4	0	1.0	0	2.42[d]
	0.06	2158	20.15	4.54	2.59	27.3[e]	0.3	0	0.51	0	0.85[e]
24 h	Control	2153	25.82	5.48	2.69	34.0[a]	0	0	0	0	0[a]
	0.5	2126	1.36	0.42	0.23	2.02[b]	9.3	2.3	9.3	4.65	25.5[b]
	0.25	2112	9.13	1.04	0.61	10.8[c]	4.8	0	2.19	0	7.01[c]
	0.125	2098	17.44	2.76	1.81	22.0[d]	2.16	0	0.65	0	2.81[d]
	0.06	2103	20.01	4.99	2.28	27.3[e]	0.52	0	0.52	0	0.52[e]

*Means with the same letters do not significantly differ at 0.05 level (One-Way Anova Test, Tukey, SPSS-21)

Table 4. Cytogenetic analysis of *A. cepa* L. root tips exposed to different concentrations of Goji berry water extracts for different periods

Time of treatment	Doses (gr / 100 ml)	Examined total cells	Prophase (%)	Metaphase (%)	Anaphase–Telophase (%)	Mitotic index (%)*	C-mitosis (%)	Anaphase bridges (%)	Stickiness (%)	Laggards (%)	Total abnormalities (%)*
12 h	Control	2208	23.64	5.16	2.85	31.7[a]	0	0	0	0	0[a]
	0.5	2213	19.79	4.47	2.21	26.5[b]	0.34	0	0.51	0	0.85[b]
	0.25	2106	23.02	4.79	2.42	30.2[c]	0	0	0.16	0	0.16[c]
	0.125	2198	22.56	5.09	2.63	30.3[c]	0	0	0	0	0[a]
	0.06	2110	23.79	5.35	2.93	31.1[d]	0	0	0	0	0[a]
24 h	Control	2153	25.82	5.48	2.69	34.0[a]	0	0	0	0	0[a]
	0.5	2121	20.98	4.52	2.02	27.5[b]	0.17	0	0.34	0	0.51[b]
	0.25	2157	21.69	4.58	2.13	28.4[c]	0	0	0	0	0[a]
	0.125	2146	23.67	4.93	2.42	31.0[d]	0	0	0	0	0[a]
	0.06	2128	24.71	5.16	2.58	32.5[e]	0	0	0	0	0[a]

*Means with the same letters do not significantly differ at 0.05 level (One-Way Anova Test, Tukey, SPSS-21)

DISCUSSION

In this study we examined the effects of Goji berry water and ethanol extracts on fertility and physical growth of *C. Elegans.*, a statistically significant dose-related decrease was determined in the fertility of *C. elegans* with ethanol extract compared to the water extract Fertility was reduced with high doses of water extracts, while an increase was determined at the lowest dose used in the study.

The difference in the findings of ethanol extract at all doses compared to the control group were statistically significant. When the effects of the Goji berry water and ethanol extracts on newly-hatched L1 form *C. elegans* were examined, a statistically significant retardation in growth was determined in the first 3 doses of ethanol extract (0.5 gr/100mL, 0.25 gr/100mL, 0.125 gr/100mL) compared to the control group, while no significant difference was found at the dose of 0.06gr/100mL. In the experiment with water extract, no significant differences was determined at any doses.

There are several studies in literature related to Goji berry, most of which have examined the antioxidant effects of the fruit extracts. A consensus has been reached that this fruit has a strong antioxidant effect through the suppression of free oxygen radicals [13-15].

In the ageing process, the anatomic and functional degeneration observed in organs is known to be caused by reduced antioxidant capacity and damage formed by free radicals. This anti-oxidant property is thought to be associated with the protective effect shown directly through the neutralisation of oxygen-origin free radicals and indirectly by activating antioxidant enzymes or inhibiting pro-oxidative enzymes. In this context, the programmed cell death, known as apoptosis, is assisted by increasing the antioxidant capacity of the body and it is thought that the formation of cancer could be inhibited in this way. Therefore, the anti-ageing and anti-oxidant effects of Goji berry have been discussed in literature [16-20]. There has been also frequent mention of the neuroprotective effects of Goji berry [6, 21], the cytoprotective effects [22], and the regulatory effects on serum lipid levels [23].

When the methods of the majority of these studies are examined, it can be seen that the polysaccharides were extracted by drying the Goji berry fruit, rendering it into powdered form then boiling in water. In the water extract findings of the current study, there were seen to be positive effects at low doses. At the 0.06gr/100mL dose in particular, an increase was seen in the productivity of eggs in the model organism. It is thought that the effect could be greater at lower doses. With oral intake by living creatures, this food substance is exposed to to acid in the stomach and to several digestive enzymes presented in the gastro-intestinal system of the body. Therefore, in contrast to *in-vitro* studies in particular, by exposure to many different substances during digestion the effect may be very different. In the current study, the findings of the water extract and the ethanol extract were different and the negative effect of ethanol extract was much greater.

In previous studies, plant polysaccharides have been reported to have stimulating effects on male fertility [24, 25]. However, there has been no study which has investigated the effects of Goji berry on egg count or physical growth in a female or hermaphrodite model organism.

In a study made on the reproductive effects of Goji berry on males, molecular evaluation was made of male rats after treatment with H_2O_2. At the end of the study, testis cells were evaluated with comet analysis and a protective effect was observed on the cell DNA. The greatest protective effect was determined at the lowest dose used in the study of 50µg/mL and the protective effect decreased as the dose increased. In the same study, after the testes of the male rats were treated with heat, testosterone, FSH and LH levels were measured. Testosterone was measured at 27.05 nmol/L at a dose of 10 mg/kg per day and at 17.21 nmol/L at a dose of 200 mg/kg per day. In the control group with no heat application this value was 16.09 nmol/L. In the groups administered with Goji berry lipopolysaccharides, a dose-related increase was determined in the FSH and LH hormone levels, with the highest hormone level determined at the lowest dose [26].

In the current study, the effects of the extracts were tested on the model organism in which no stress had been created. The genotoxic effects of the ethanol extracts were seen to be high and it was especially noticeable that C-mitosis was greater in the cells. This is associated with spindle threads not being formed in the chromosomes distributed in the metaphase or with deterioration of the structure, thereby preventing cell division. In addition, cell division was observed to be significantly inhibited by the ethanol extract in particular, which was thought to be due to the association of the mitotic index to the dose. Again there were no chromosome fractures and the low number of laggard chromosomes were seen to be in parallel with the results of comet analysis as mentioned above. Comet analysis is known to be an analysis method which tests chromosome damage with the formation of a tail in the electromagnetic field of DNA, based on the examination of DNA damage at the level of a single cell, such as chromosome fractures or laggard chromosome.

The findings obtained in the current study developed according to the dose. It is thought that the effect could be lower at lower doses. It was noticeable that the genotoxic effect was very low in the water extract. The difference between the findings of the test made with the water extract and the ethanol extract was thought to possibly originate from the greater concentration of polysaccharides in the ethanol extract. The findings of a study by Cai et al. [27] confirmed the findings of the current study. In that study, the Goji berry was analysed with the HPLC method and total phenolic substance was determined at the rate of 2.58 % (g/100gr dry weight) in methanol extract and at 0.70% (g/100 gr dry weight) in water extract. As a result of that study, the substance found weighted within the amount of phenolic substance was determined as scopoletin from coumarins [27]. Scopoletin is known to have antibacterial, anti-inflammatory, antiseptic, bronchorelaxant, anti-asthmatic and cancer preventive effects. Previous studies have also determined that this substance has a

regulatory role in hyperthyroidism and hypereglycemia, inhibits lipd peroxidisation and increases anti-oxidant activity [28].

Cell division which provides continuation without changing the chromosome series in an organism, is known as mitosis. Mitotic division occurs in two division phases of nucleus division and cytoplasm division. In multiple cell organisms, mitotic division from proliferation of cells forming the soma, or body, is known as somatic division [29]. Mitotic division is extremely important for the continuation of life with several functions in the body from cell renewal to wound healing. In addition, the importance of mitosis is extremely high in pregnancy. Approximately 30 hours after impregnation, the first mitotic division occurs for a zygote to become an organism with two cells. During mitotic division, the inherited material in the divided cell is passed in equal amounts to the divided cells. This dividing and multiplying process continues rapidly throughout the germination period. The development of a fetus in the womb is provided by this germination stage from a single cell organism. For a zygote to become multi-celled, it rapidly reproduces for approxiamately 72 hours. This stage is generally known as the morula. Then cell division develops rapidly for 4 days and the cells, known as blastocytes form an aggregation. At this stage, the fetus can easily settle in the uterus. After the zygote has divided into two, the two-cell stage is reached and this is the first step in the life of an infant. After 2 cells, there are 4 cells and this division continues. Only in the 3rd week of pregnancy does cell division continue with growth. Thus it can be seen that mitotic division demands importance from the very beginning of life. Therefore, substances which reduce cell division, lowering the mitotic index can constitute a risk to the fetus more at the impregnation stage. In the current study, Goji berry ethanol extract significantly reduced the mitotic index in a dose-dependent manner.

In conclusion, there are many pharmaceutical active substances that have been isolated from plants. It must not be ignored that the uncontrolled use of medicinal plants could result in side-effects in the body. Goji berry, which has been used in Chinese medicine since ancient times and for which several advantages have been mentioned in literature, was seen in the current study to have negative effects on cell division, fertility and physical growth, which emphasises the importance of this subject. In Turkey, Goji berry is often used because of the weight-loss and anti-diabetic effects and the results of the current study showed that there could be harmful effects above a certain dose. These effects may be seen as low egg production in young females and growth retardation in childhood. As the use of Goji berry reduces mitotic cell division, this could be a particular problem in pregnancy. It must also not be ignored that these effects could be increased with alcohol intake. As there is uninformed usage by the public, the sale and easy availability of medicinal plants such as this should be brought under control to benefit public health.

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REFERENCES

1. Amagase H, Farnsworth NR. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of Lycium barbarum fruit (Goji). Food Res Int. 2011;44:1702-7.
2. Bryan JK, Casta D, Giese N, Nummy K, Rapp C, Seamon E. Goji (Lyciumspp) in natural standard monograph. 2008. Natural Standard Inc.
3. Dag S, Ozpinar H, Sari M, Ozpinar N. Antimicrobial effect of cola on several microorganisms. Cumhuriyet Sci J. 2015;36 52-9.
4. Potterat O. Goji (Lycium barbarum and L. chinense): phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med. 2010;76 7-19.
5. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001;109:69-78.
6. Chan HC, Chang RCC, Ip AKC, et al. Neuroprotective effects of Lycium barbarum Lynn on protecting retinal ganglion cells in an ocular hypertension model of glaucoma. Expe Neurol. 2007;203:269-73.
7. Chalasani SH, Chronis N, Tsunozaki M, Gray JM, Ramot D, Goodman MB, et al. Corrigendum: Dissecting a circuit for olfactory behaviour in Caenorhabditis elegans. Nature. 2016;533:130
8. Ono K, Ono S. Two distinct myosin II populations coordinate ovulatory contraction of the myoepithelial sheath in the Caenorhabditis elegans somatic gonad. Mol Biol Ccell. 2016:27:1131-42.
9. Ozpinar H, Ozpinar N, Kilicgun H, Dag S, Sari M. Physiological and lifespan alterations in Caenorhabditis elegans exposed to Benzoic acid. SSTB. 2011;7:39-47.
10. Olgun A, Aleksenko T, Pereira-Smith OM, Vassilatis DK. Functional analysis of MRG-1: The ortholog of human MRG15 in Caenorhabditis elegans. J Gerontol A Biol Sci Med Sci. 2005;60:543-8.
11. Turkoglu S. Genotoxicity of five food preservatives tested on root tips of Allium cepa L. Mutat Res Genet Toxicol Environ Mutagen. 2007;626:4-14.
12. Koelle MR, Horvitz HR. EGL-10 regulates G protein signaling in the C. elegans nervous system and shares a conserved domain with many mammalian proteins. Cell. 1996;84:115-25.
13. Li X, Li X, Zhou A. Evaluation of antioxidant activity of the polysaccharides extracted from Lycium barbarum fruits in vitro. Eur Polym J. 2007;43:488-97.
14. Lin CL, Wang CC, Chang SC, Inbaraj BS, Chen BH. Antioxidative activity of polysaccharide fractions isolated from Lycium barbarum Linnaeus. Int j biol Macromol. 2009;45:146-51.
15. Wu SJ, Ng LT, Lin CC. Antioxidant activities of some common ingredients of traditional chinese medicine, Angelica sinensis, Lycium barbarum and Poria cocos. Phytother Res. 2004;18:1008-12.
16. Chang RCC, So KF. Use of anti-aging herbal medicine, Lycium barbarum, against aging-associated diseases. What do we know so far?. Cell Mol Neurobiol. 2008;28:643-52.
17. Gan L, Zhang SH, Yang XL, Xu HB. Immunomodulation and antitumor activity by a polysaccharide–protein complex from Lycium barbarum. Int Immunopharmacol. 2004;4:563-9.
18. Li P, Xiao B, Chen H, Guo J. Lycium barbarum and tumors in the gastrointestinal tract, in Lycium barbarum and human health. 2015. Springer Netherlands, 2015. p. 85-97.
19. Mao F, Xiao B, Jiang Z, Zhao J, Huang X, Guo J. Anticancer effect of Lycium barbarum polysaccharides on colon cancer cells involves G0/G1 phase arrest. Med Oncol. 2011;28:121-6.
20. Yu MS, Lai CSW, Ho YS, Zee SY, So KF, Yuen WH, el al . Characterization of the effects of anti-aging medicine Fructus lycii on ß-amyloid peptide neurotoxicity. Int J Mol Med. 2007;20:261-8.

21. Yu MS, Leung SKY, Lai SW, Che CM, Zee SY, So KF, et al. Neuroprotective effects of anti-aging oriental medicine Lycium barbarum against β-amyloid peptide neurotoxicity. Exp Gerontol. 2005;40:716-27.
22. Yu MS, Ho YS, So KF, Yuen WH, Chang RCC. Cytoprotective effects of Lycium barbarum against reducing stress on endoplasmic reticulum. Int J Mol Med. 2006;17:1157-62.
23. Ming M, Guanhua L, Zhanhai Y, Guang C, Xuan Z. Effect of the Lycium barbarum polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo. Food Chem. 2009;113:872-7.
24. Mazaro R, Di Stasi LC, Kempinas WDG. Effects of the hydromethanolic extract of Austroplenckia populnea (Celastraceae) on reproductive parameters of male rats. Contraception. 2002;66:205-9.
25. Wang Y, Zhao H, Sheng X, Gambino PE, Costello B, Bojanowski K. Protective effect of Fructus lycii polysaccharides against time and hyperthermia-induced damage in cultured seminiferous epithelium. J Ethnopharmacol. 2002;82:169-75.
26. Luo Q, Li Z, Huang X, Yan J, Zhang S, Cai YZ. Lycium barbarum polysaccharides: Protective effects against heat-induced damage of rat testes and H_2O_2 induced DNA damage in mouse testicular cells and beneficial effect on sexual behavior and reproductive function of hemicastrated rats. Life Sci. 2006;79:613-21.
27. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74:2157-84.
28. Panda S, Kar A. Evaluation of the antithyroid, antioxidative and antihyperglycemic activity of scopoletin from Aegle marmelos leaves in hyperthyroid rats. Phytother Res. 2006;20:1103-5.
29. Topaktas M, Rencuzogullari E. Cytogenetic. 2010: Nobel publishing. 22-23.