Protective effects of BioZen chip against mobile phone radiation on the model of developing quail embryo

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Aim: To assess the effect of BioZen chip on biological activity of mobile phone radiation.

Materials and Methods:

Biological model: Quail embryos *in ovo.* Fresh hatching eggs from the local producer.
Mobile phones: Commercial models of Huawei 5YII, new (GSM 1800 MHz), from the local mobile phone shop.
BioZen chip: New, from the producer.
Chemical supplies: Applicable for biochemical tests, mostly from Sigma Aldrich.
Incubators: Plastic-foam, automatic thermoregulation, made specially for the EMF tests, tested before.
MW meter: RF field strength meter (Alfalab, USA).
Other lab equipment: spectrophotometer Specol 80 (Germany), ESR spectrometer RE 1307 (Russia).

Test design: For each test, three analog groups of fresh hatching eggs (n=10-15) were formed: 1) as unprocessed control (C); 2) exposed to EMR from the mobile phone (H), fig 1; 3) exposed to EMR from the mobile phone with attached BioZen chip (H+W), fig 2, 3. The exposure of groups H and H+W were carried out during 5 days before the incubation (under the room temperature) and during 14 days of incubation, totally 19 days. Mobile phones were situated 3 cm over the hatching egg surface back side of phone to the eggs. Mobile phone exposure was activated due to computer auto-redial system (48 c – On; 48 c – Off) for each phone. Before the incubation the groups were kept separately at least 2 m from each other. The incubation was carried out in three different incubators, separately for each group. The distance between the incubators was at least 2 m. The conditions of incubation and keeping the eggs before the incubation were equal for three

groups, excluding the relevant exposure for group H and group H+W. The parameter of incubation was close to optimal.
There were three terms of analysis: 38 hour embryos, 10 days embryos; and one day old chicks (after 17 days of incubation).

The embryos and chicks were tested on:

- the intensity of somitogenesis (38 h embryos);
- level of thiobarbituric acid reactive substances (TBA-RS), indicator of lipid peroxides level (38 h embryos);
- antioxidant enzymes activities (catalase; superoxide dismutase/SOD; and ceruloplasmin) (38 h embryos);
- double strand breaks of DNA (38 h embryos);
- superoxide and nitrogen oxide production (10 day embryos, one day chicks);
- level of 8-oxo-dG, marker of oxidative damages of DNA (one day chicks).

Analysis of somitogenesis, tests on TBA-RS and antioxidant enzyme activities were used as described in [1]. Ceruloplasmin test is described in [2]. Level of DNA double strand breaks in comet assay was assessed as in [3]. Levels of superoxide and nitrogen oxide production were assessed in spin-traps ESR technique [4]. Level of 8-ozo-dG was assessed as described in [4]. Statistical analysis was carried out according to t student test.

The intensities of microwaves (MW) from mobile phones and MW background were checked daily.

Results:

MW intensity from the mobile phones varied significantly during the time of exposure, from 0.05 to 20 μW/cm^2 but correlated between mobile phones that indicates on external reason, for example change in operation state of the nearest base transceiver station. Nevertheless, the average intensity of MW from the mobile phones, 0,323±0,054 μW/cm^2 was far below the official safety limits, 450 μW/cm^2 for the most European countries. There was not detected the difference in intensity of MW from mobile phones without and with BioZen chip. The background MW in the lab was about 0.001 μW/cm^2.

38 hour embryos:

Intensity of somitogenesis was statistically significantly increased under mobile phone radiation exposure (group H). It means a number of differentiated somite pairs was 13.4% higher in H group as compared to the control (table 1, fig 4-6). BioZen application normalized the effect of mobile phone exposure, returning the number of differentiated somites to the control level. The difference between group H and group H+W was statistically significant ($p<0.05$).

DNA double strand breaks detected in alkaline comet assay was statistically significantly (17.5%) higher in embryonic cells exposed to mobile phone radiation as compared to control (table 1, fig. 7-9). At the same time, in cells of embryos exposed to mobile phone with BioZen chip level of DNA damages was on the same level as in control embryos.

Oxidative effect. Mobile phone exposure produced significant oxidative effect in the embryo cells (group H): level of TBA-RS (lipid peroxides) was significantly higher as compared to the control, 61.1%, $p<0.05$ (table 1), ceruloplasmin activity in this group was 110% ($p<0.05$) higher than in control, and catalase activity was 60.2% higher than in control (although the last difference was not statistically significant).

BioZen chip applied to the mobile phone statistically significantly normalized oxidative status of exposed embryo cells (group H+W): TBA-RS level, activities of ceruloplasmin and catalase were close to control. Thus, level of TBA-RS, and activity of ceruloplasmin in H+W group were significantly ($p<0.05$-0.001) lower than in H group (table 1). Activities of SOD were not significantly changed in both exposed groups and thus were close to control.

Ten day embryos:

Superoxide radical generation was statistically significantly, 140 – 210%, increased in cells of brain, heart and liver of 10 day embryos exposed to mobile phone radiation as compared to the control (table 2). The same level of superoxide generation was detected in group of 10 day embryos exposed to mobile phone with BioZen chip radiation.

Nitrogen oxide generation also statistically significantly increased in cells of 10 day embryos after mobile phone radiation exposure, 18.7 – 84.7%, as compared to the control (table 2). Approximately the same level of nitrogen oxide generation had place in cells of 10 day embryos exposed to mobile phone with BioZen emission. The only cells of heart

of this group of embryos had 33.4% lower generation of nitrogen oxide as compared to mobile phone group, but difference between the groups was not statistically significant.

One day old quail chicks:

Hatchability (percent of chicks obtained from fertilized eggs) was statistically significantly ($p<0.05$) decreased in group of eggs/embryos exposed to mobile phone radiation as compared to the control (20% against 57.9%). Thus, mobile phone radiation resulted in significant increase of embryo mortality. Hatchability of eggs/embryos exposed to mobile phone with BioZen radiation was slightly higher than in mobile phone group (28.6% against 20%), although still much lower than in control.

Superoxide generation was significantly, 180-217%, increased in cells of brain, heart and liver of one day chicks from the embryos exposed to mobile phone radiation (table 3). At the same time, chicks from the embryos exposed to mobile phone with BioZen emission demonstrated significantly less activation of superoxide generation, 48.3-99.6% less as compared to the mobile phone group. Although the indexes were still statistically significantly higher than in control, they were much closer to the control than the indexes of mobile phone group.

Nitrogen oxide level in cells of brain, heart and liver of one day chicks from mobile phone exposed embryos was 25-87.7% higher than in control. The differences with control are statistically significant for all organs (table 3). On the other hand, application of BioZen chip to mobile phone during the exposure resulted in statistically significant decrease of nitrogen oxide level (14.7-16.9%) in brain and heard of chicks as compared to the chicks from mobile phone exposed embryos.

8-oxo-dG level, a marker of oxidative damages of DNA, statistically significantly, 63.4-119.4%, increased in brain, heart and liver of one day chicks from mobile phone exposed embryos. Application of BioZen chip resulted in statistically significant decrease of 8-oxo-dG level, 73.9-74.6%, as compared to mobile phone group (table 3).

Discussion:

First, the test model, a developing quail embryo, and methods of analysis applied have allowed to demonstrate statistically significant adverse biological effects of GSM 1800 MHz radiation from commercial model of smartphone Huawei 5YII. There were detected statistically significant changes in the intensity of somitogenesis, expressive oxidative effects and damages of DNA integrity in cells of 38 hour embryos under the GSM 1800 MHz exposure in intensity three orders of magnitude lower than official “safety limits”.

The adverse effects under the mobile phone radiation exposure were persistent during all embryogenesis and included 2 fold increase in level of superoxide generation and up to 85% increase in nitrogen oxide generation in tissues of 10 day embryos and one day chicks. Also, in one day chicks there were demonstrated statistically significant oxidative damages of DNA. Finally, Huawei 5YII emission applied to quail embryos 5 days before the incubation and during 14 days of incubation resulted in significant, almost twice, increase of embryo mortality as compared to the control.

On this background, application of BioZen chip for modulation of mobile phone radiation resulted in statistically significant normalizing effect on metabolism in exposed embryonic cells. There were detected statistically significant normalization of the intensity of somitogenesis, oxidative status of embryonic cells and integrity of DNA in 38 hour embryos. Also, statistically significant normalization in superoxide and nitrogen oxide production in tissues of one day chicks had place. It is important that also statistically significant decrease in oxidative damages of DNA (level of 8-oxo-dG) was detected in this group of chicks as compared to mobile phone only exposed embryos. Although indexes of free radical generation and oxidative damages of DNA after application of BioZen chip did not return exactly to the control level, they had been much closer to control levels than indexes of mobile phone group. Also, slight decrease of embryo mortality in mobile phone + BioZen group as compared to mobile group of embryos was detected.

It is interesting that application of BioZen chip to mobile phone did not reveal significant normalizing effect in 10 day exposed embryos, in a period of intensive embryo growth and significant disorder in superoxide and nitrogen oxide production due to mobile phone exposure. But critically important outcome is that even under such strong oxidative stress conditions normalizing effect of BioZen on mobile phone radiation was detected in a few days, at the end of embryogenesis.

In conclusion, statistically significant persistent oxidative stress and mutagenic effects in embryonic cells, as well as increased embryo mortality have been detected under such low intensity of GSM 1800 MHz as 0.3 μW/cm^2 on the model of developing quail embryos. Application of BioZen chip to mobile phone Huawei 5YII during the exposure resulted in normalizing/protective effect on oxidative status and DNA integrity in embryonic cells. These findings allow recommending BioZen chip as a promising approach to reducing adverse effects of mobile phone radiation for human health.

References

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Table 1. Intensity of somitogenesis, damages of DNA and oxidative status of living cells of **38-h quail embryos** after exposure to mobile phone Huawei 5YII or mobile phone Huawei 5YII + BioZen chip emission (n=7; M ± m)

Index	Control group	Huawei 5YII group	Huawei 5YII + BioZen chip
Differentiated pairs of somites, n	10.86 ± 0.34	12.38 ± 0.46*	11.13 ± 0.4 #
DNA double strand breaks (% of DNA in tail of comet)	14.3 ± 0.71	16.96 ± 1.13*	14.56 ± 0.96
TBA-RS, μmol/g	0.72 ± 0.06	1.16 ± 0.17*	0.78 ± 0.06 #
Catalase, ncat/g	5.88 ± 0.68	9.42 ± 1.66	7.17 ± 1.92
Ceruloplasmin, mg/100 ml	4.52 ± 0.75	9.53 ± 0.75*	4.2 ± 0.58 ###
SOD, rel.un.	0.147 ± 0.03	0.159 ± 0.02	0.162 ± 0.016

Here and below:
* - $p<0.05$ as compared to control; ** - $p<0.01$ as compared to control; *** - $p<0.001$ as compared to control;
# - $p<0.05$ as compared to H group; ## - $p<0.01$ as compared to H group; ### - $p<0.001$ as compared to H group;

Table 2. Generation of superoxide and nitrogen oxide in cells of **10-day quail embryos** after the exposure to mobile phone Huawei 5YII or mobile phone Huawei 5YII + BioZen chip emission (n=7; M ± m)

Index	Control group	Huawei 5YII group	Huawei 5YII + BioZen chip
Superoxide (nmol/g):			
- in brain;	0.24 ± 0.009	0.578 ± 0.1*	0.573 ± 0.082 **
- in heart;	0.24 ± 0.009	0.754 ± 0.078***	0.87 ± 0.114 ***
- in liver;	0.22 ± 0.016	0.586 ± 0.049***	0.588 ± 0.101 **
Nitrogen oxide (nmol/g):			
- in brain;	1.51 ± 0.037	1.796 ± 0.081**	1.798 ± 0.099*
- in heart;	1.50 ± 0.029	2.77 ± 0.174***	2.27 ± 0.128***
- in liver;	1.48 ± 0.025	2.61 ± 0.173***	2.58 ± 0.133***

Table 3. Generation of superoxide and nitrogen oxide, and level of 8-oxo-dG in cells of **1-day quail chicks** after the exposure of embryos to mobile phone Huawei 5YII or mobile phone Huawei 5YII + BioZen chip emission (n=5; M ± m)

Index	Control group	Huawei 5YII group	Huawei 5YII + BioZen chip
Superoxide (nmol/g):			
- in brain;	0.23 ± 0.01	0.73 ± 0.05***	0.50 ± 0.08 ##
- in heart;	0.29 ± 0.01	0.83 ± 0.05***	0.69 ± 0.05
- in liver;	0.26 ± 0.01	0.73 ± 0.04***	0.57 ± 0.05 #
Nitrogen oxide ((nmol/g):			
- in brain;	1.42 ± 0.03	1.78 ± 0.05**	1.57 ± 0.04 #
- in heart;	1.48 ± 0.01	2.08 ± 0.03***	1.83 ± 0.06 ##
- in liver;	1.46 ± 0.02	2.74 ± 0.05***	2.54 ± 0.08
8-oxo-dG level (nmol/g):			
- in brain;	0.41 ± 0.03	0.67 ± 0.04**	0.59 ± 0.04
- in heart;	1.49 ± 0.02	3.2 ± 0.09***	2.1 ± 0.18 ###
- in liver;	0.67 ± 0.03	1.47 ± 0.05***	0.97 ± 0.05 ###

Fig 1. Huawei 5YII radiation exposed quail embryos in ovo.

Fig 2. Huawei 5YII + BioZen chip exposed quail embryos in ovo.

Fig 3. Huawei 5YII with BioZen chip.

Fig 4. Microphoto of 38-h quail embryo of control group (11 pairs of somites).

Fig 5. Microphoto of 38-h quail embryo of Huawei 5YII exposed group (13 pairs of somites).

Fig 6. Microphoto of 38-h quail embryo of Huawei 5YII + BioZen chip exposed group (11 pairs of somites).

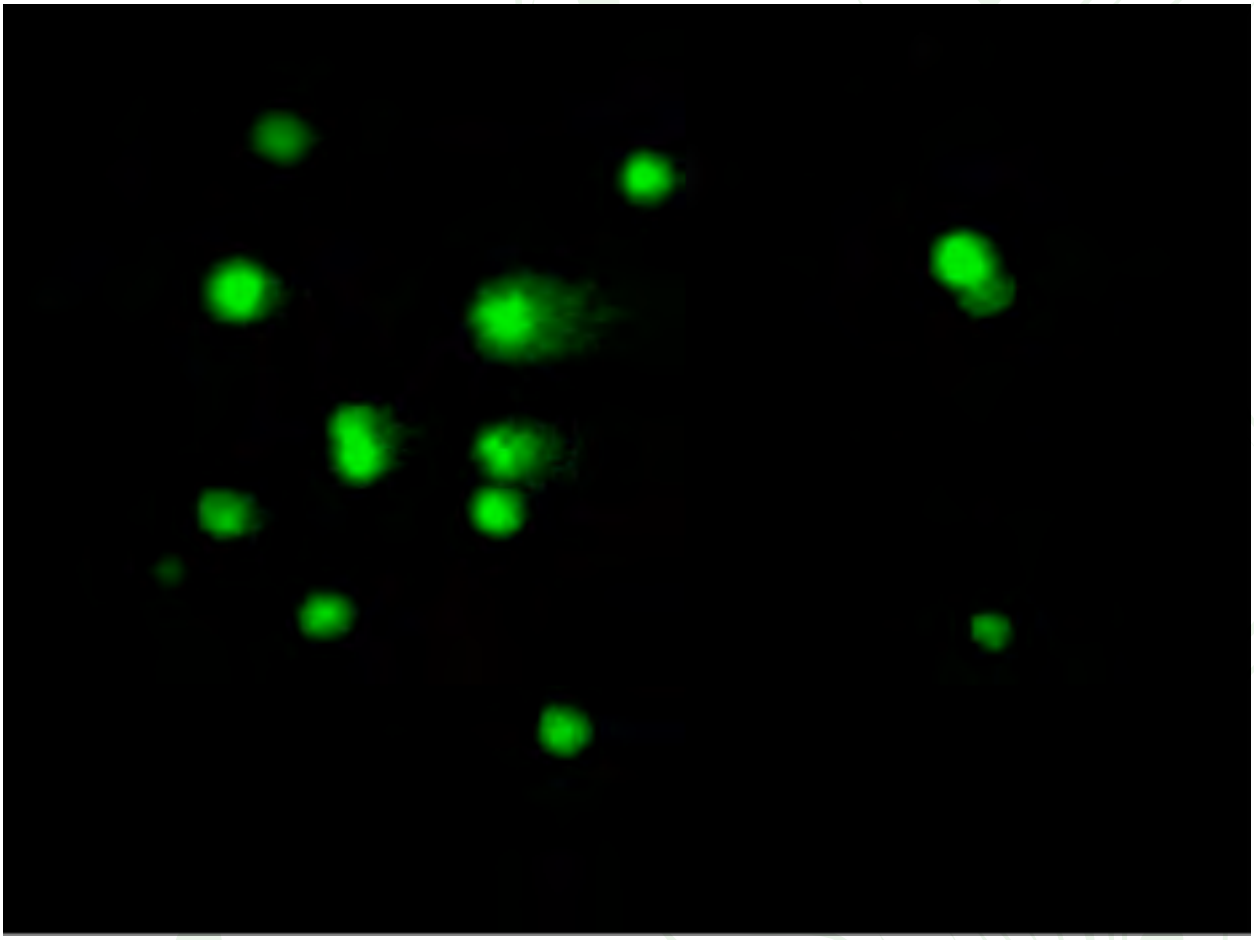

Fig 7. Microphoto of comets of 38-h quail embryo cells (control).

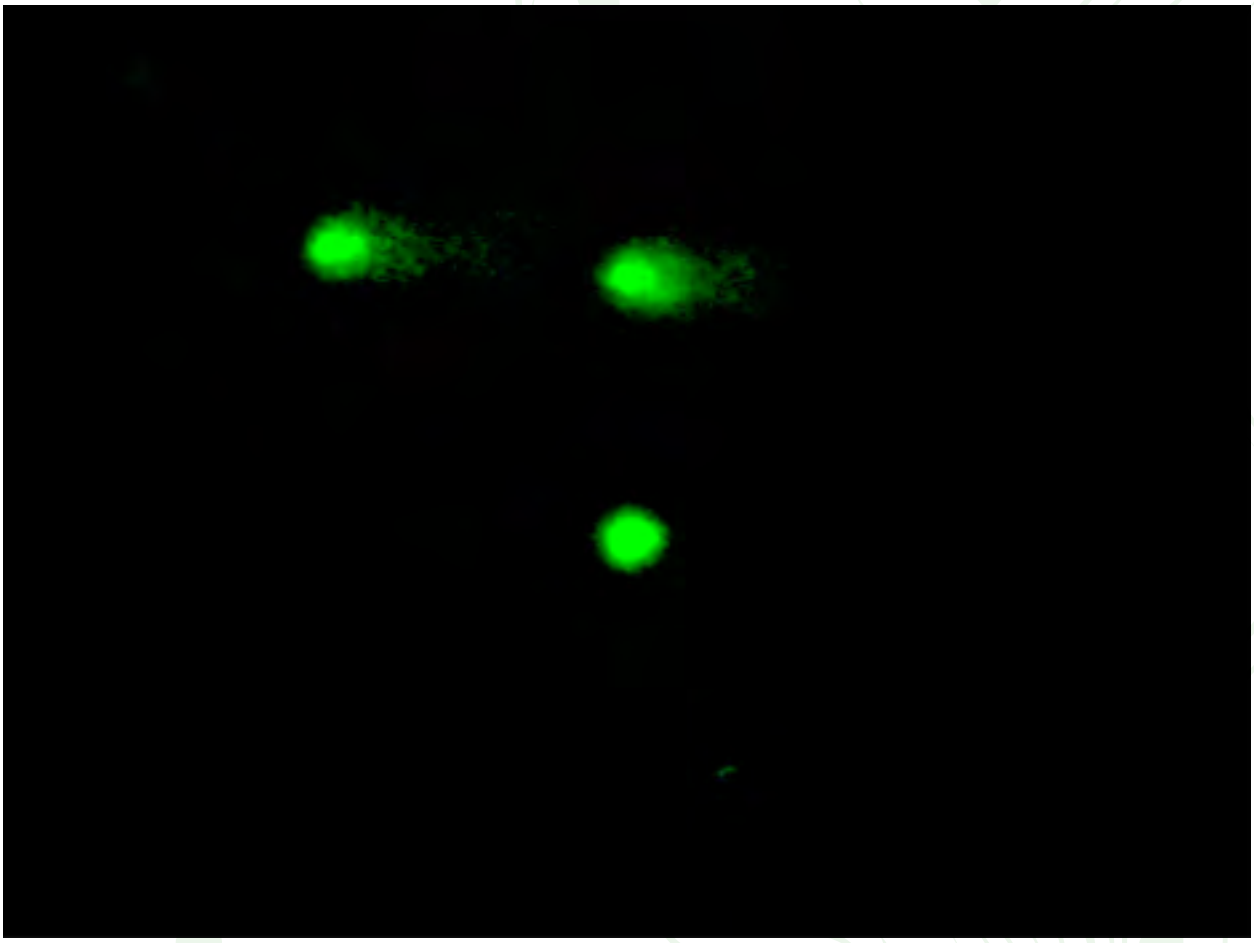

Fig 8. Microphoto of comets of 38-h quail embryo cells after Huawei 5YII radiation exposure.

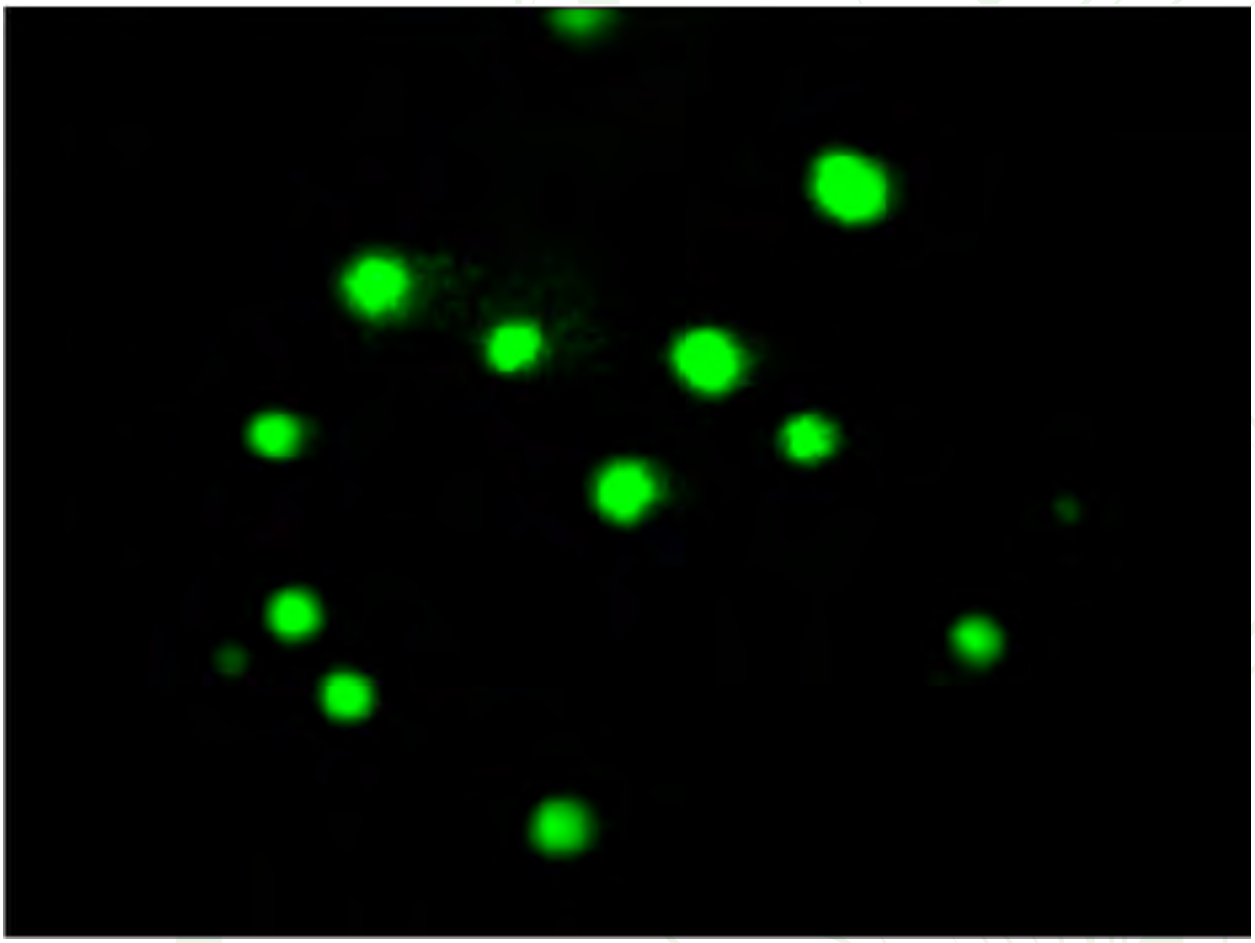

Fig 9. Microphoto of comets of 38-h quail embryo cells after Huawei 5YII + BioZen chip exposure.