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Effect of Cold Atmospheric Plasma on Ehrlich Carcinoma

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RECENT progress in atmospheric plasma has led to creation of cold plasma with ions temperature close to room temperature. In present study the effects of cold atmospheric plasma jet on Ehrlich Carcinoma (EC) has been revealed. The present study is carried out on three groups of Males Swiss albino mice bearing Ehrlich Carcinoma. Design of suitable plasma source is used for the treatment. Tumors are ablated with a single transdermal cold atmospheric plasma (CAP) treatment. Tumor volume and mice survival parameters are determined. Results indicate that the mechanism of action for cold plasma on cancer cells is due to generation of reactive oxygen species (ROS). This is occurred with possible induction of the apoptosis pathways. The results of this study reveal that the CAP greatly inhibits Ehrlich Carcinoma cells (ECC) growth. This is considered as an advanced new modality for treatment of cancer cells with least side effects.

Keywords: Cold plasma, Ehrlich carcinoma and Reactive oxygen species (ROS).

A plasma is an ionized medium that contains numerous active components including electrons and ions, free radicals, reactive molecules, and photons [1, 2]. Plasma can be categorized as either local thermodynamic (thermal) equilibrium plasma (LTE) or non-local thermodynamic (cold) equilibrium plasma (non-LTE) and it is important to note that an atmospheric plasma jet can be divided in two zones: A central zone or plasma core which is in LTE and a peripheral zone or plasma plume which is in non-LTE.

Nowadays cold atmospheric plasma (CAP) treatments are being investigated for multiple biological applications for example: tissue sterilization, blood coagulation, wound healing, tissue regeneration, dental treatment, and the treatment of various diseases, including cancer [3, 4].

Recently, some researchers concerned with plasma in medicine have studied the possible applications in cancer therapy. Some early attempts have shown that plasma exerts antitumor effects on a wide variety of cancer cells**.** If this method is compared with conventional anticancer therapies, such as ionizing radiation and chemotherapy, plasma can kill cancer cells by triggering apoptosis of cells [5-7].

This occurs with high efficiency and fewer side-effects on the surrounding healthy cells [8]. Treatment of cancer cells with plasma exposes them to reactive oxygen species (ROS) leading to apoptosis of cells at a rapid pace [9].

Ehrlich ascites carcinoma (EAC) is one of the common model example for anticancer agent [10]. This type of research was done on males and female Swiss albino mice [11].

The aim of the present work is to study the effect of CAP on Ehrlich tumor. Continuous monitoring for tumor growth is done when mice exposed to treatment.

Materials and Methods

The present part shows the characteristics of source for treatment of Cancerous cells and the mice classifications. Figure 1, shows the cold atmospheric plasma jet system which is used for treatment.

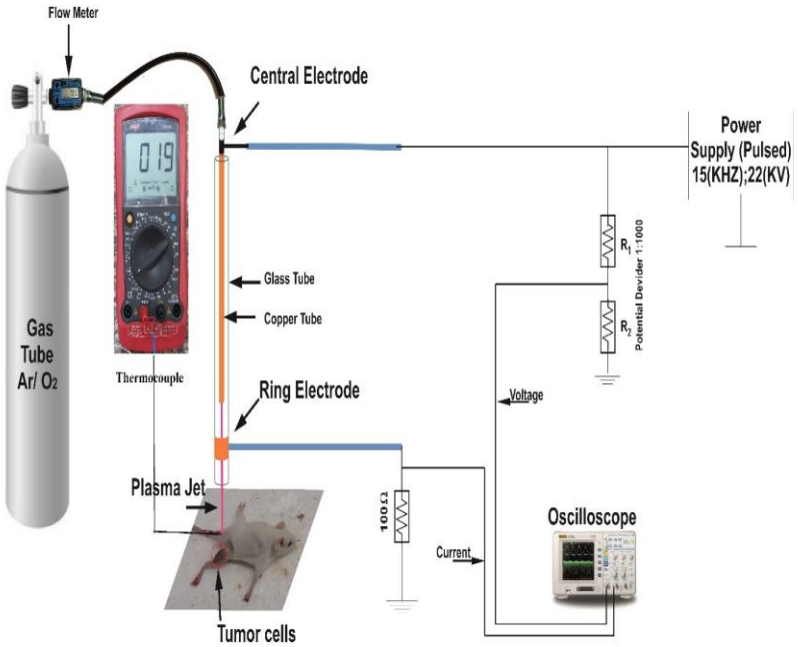

Fig. 1. Aschematic diagram of PCAPJ system for treatment of cancer cells.

The electrical characteristics of the PCAP jet discharge have been studied using the discharge circuit shown in Fig .1. The emitted jet has the following parameters as shown in Table 1.

TABLE 1. Discharge parameters of PCAP.

Gas type	**Argon**
Gas flow rate	4 l/min
Frequency	15 kHz
Discharge current	70 mA
Discharge voltage	4.5 kV
Power	3.4 W
Dose of Plasma for 2 min	6 J/cm^2
Dose of Plasma for 3 min	9 J/cm^2
Streamer length	2.3 cm
Streamer radius	0.5 cm

Spectroscopic measurements for PCAP.

Figure 2. shows the emitted Argon (Ar) lines and nitrogen (N_2) bands. Oxygen (O) line at (777.4 nm) and hydroxyl group (OH) at (308nm) are observed due to the presence of argon and air. OH and O species are known for their antimicrobial action due to their high oxidation potentials. These data are in agreement with those obtained by García-Alcantara *et al.* and Dong *et al.* [12,13].

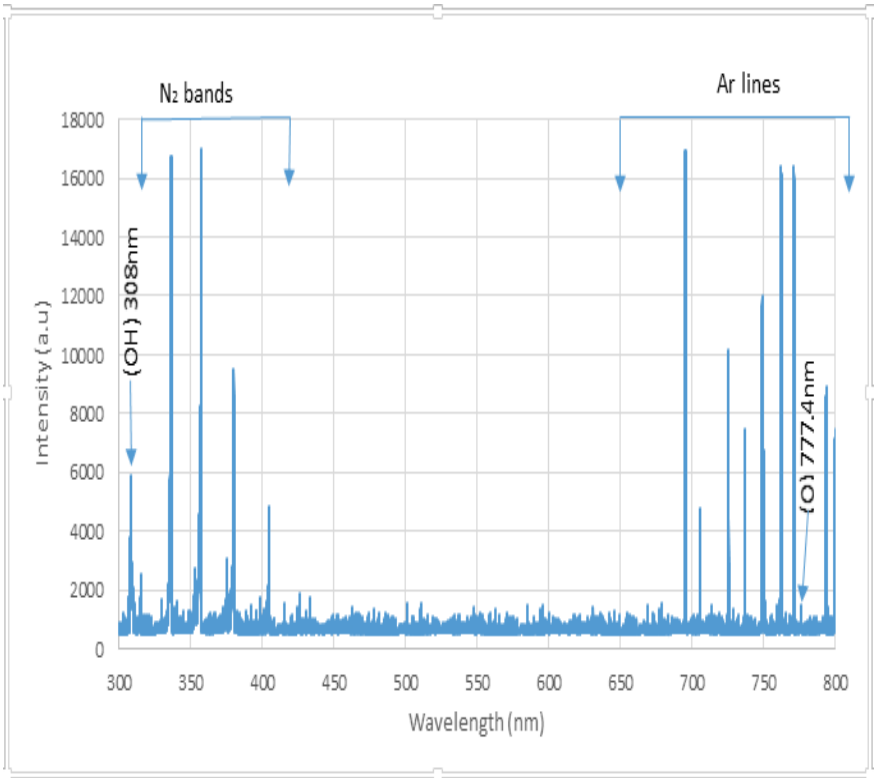

Fig. 2. Emission spectra of Ar plasma jet at 300-800 nm. Applied voltage = 4.5 kV, gas flow rate =4l/m and position of plasma jet = 1 cm.

Thermal properties along axial jet

Figure 3, indicates the dependence of temperature on the axial distance outside the glass tube which is determined by thermocouple device along the outside discharge at various distances from the end of tube. This temperature is decreasing from about 80°C at 0.5 cm distance to 37°C at 2.3 cm from the end of tube. These results are in good agreement with previously reported data [14].

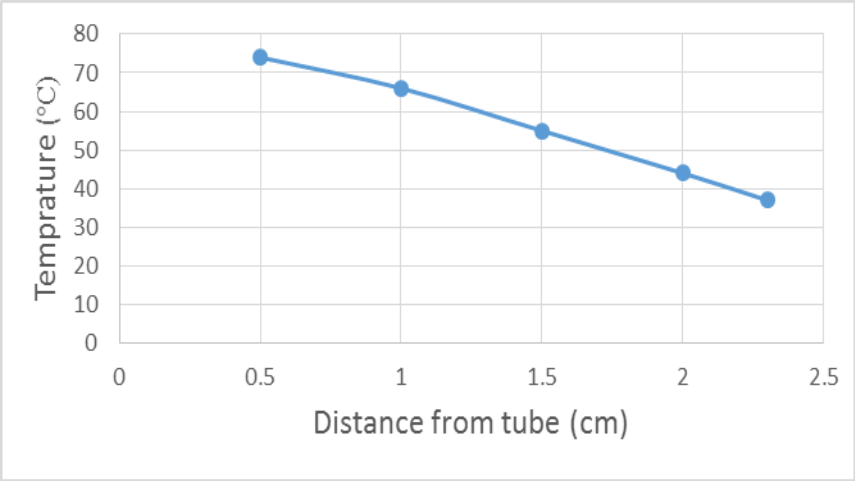

Fig. 3. Dependence of temperature on the axial distance between sensor and the end of tube.

Cell culture and tumor inoculation

Ehrlich ascites tumor is chosen as a rapidly growing experimental tumor model [15] where various experimental designs for anticancer agents can be applied. Ehrlich ascites carcinoma cells obtained from national cancer institute "NCI", Cairo University. Animals are injected in the right thigh of the hind limb by 0.2 ml from suspension containing 1×10^6 of the Ehrlich carcinoma cells (ECC). A palpable solid tumor mass (about $\geq$100 mm^3) is developed within 12 days [16, 17]. Tumor growth is monitored post-inoculation until the desired volume is reached.

Classification of animals

The animals are divided into 3 equal groups of seven mice for each:

Group (1): control or untreated, Ehrlich tumor cells are implanted subcutaneously into the right thigh of the hind limb of mice without exposing to plasma jet.

Group (2): mice with Ehrlich tumor cells are exposed to plasma jet for 2 min, (Flow rate= 4 l/min, applied voltage =4.5 kV, plasma dose =6 J/cm^2).

Group (3): mice with Ehrlich tumor cells are exposed to plasma jet for 3 min, (Flow rate= 4 l/min, applied voltage =4.5 kV, plasma dose =9 J/cm^2).

Tumor volume estimation

Tumor volume is measured on days 12, 14, 16, 18 until 24 day after implantation of (ECC) using a digital calliper. The following formula is used to calculate the volume of the developed tumor [5].

$$\text{Tumor volume } (mm^3) = V = .52 \times (A \times B^2) \ldots\ldots\ldots \quad (1)$$

where A is the long axis of tumor, B is short axis of tumor and $\pi = 3.14$. Mice are selected for treatment when the tumor reaches the desired volume. Finally all mice are sacrificed. The tumors are excised for histopathological examination.

Statistical analysis

Data are presented as mean ± SE, data are analyzed by using student t-test and difference between the mean of different groups is considered significant at a level of $p < 0.05$.The statistical program applied is Statistical Package for the Social Sciences (SPSS).

Results and Discussion

The advancement of tumor are followed thoroughly through experimental measurements. Various means of analysis are also performed.

Inhibition of tumor growth by influence of PCAP

The effect of cold plasma *in-vivo*, when the cold plasma jet is applied to mice which injected with Ehrlich ascites carcinoma. Figure 4A shows the tumor formed by injection. It is observed that a single cold plasma treatment through overlying skin leads to tumor ablation where tumor volume diminishes and doesn't grow again as shown as Fig. 4B. This procedure is applied on 2 groups, each contains 7 mice. The first group is treated for a period of 2 min using dose of pulsed cold atmospheric plasma (PCAP) of 6 J/cm^2 and the second one for 3 min using dose of PCAP of 9 J/ cm^2.

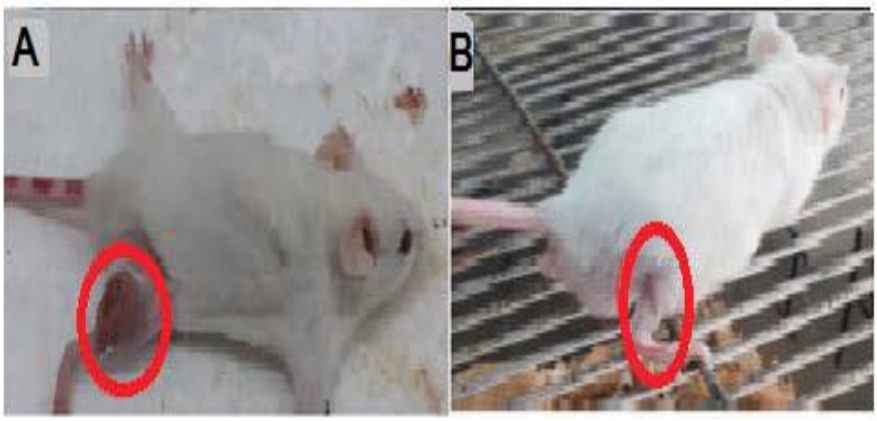

Fig. 4 A. Mice with clear tumor, B. Mice without tumor after treatment.

As shown in Fig. 5, rates of tumor growth are markedly decreased after cold plasma treatment.

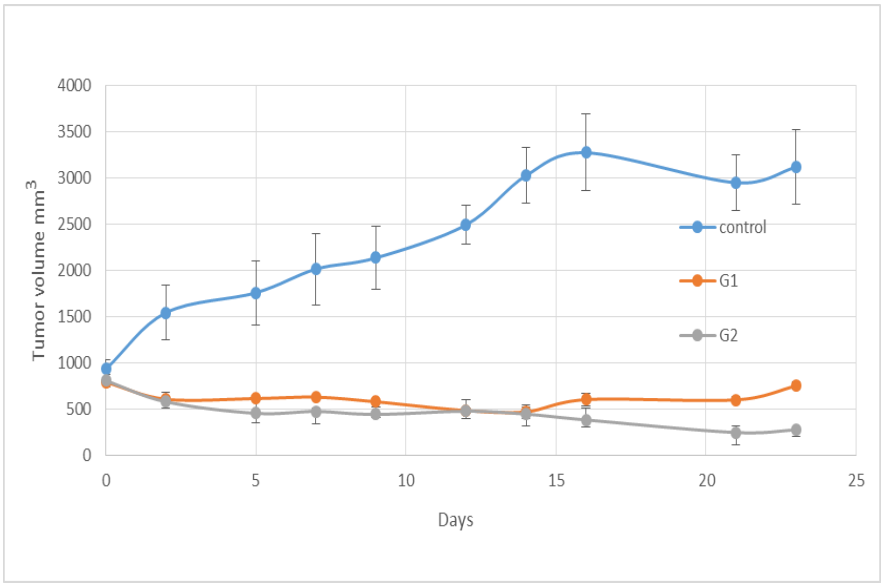

Fig. 5. Effect of PCAPJ on tumor volume.

Table 2 indicates the effect of PCAP on tumor volume. It is noticed that the average tumor volume increases in the control group from 929.6±100 to 3116.6±406 mm^3 after 23 days, although the average tumor volume of the first group decreases significantly from 788±38.5 to 748±41 mm^3 and the average tumor volume of the second group decrease from 805.9±71 to 273±26.45mm^3 after 23 days. This shows that the inhibition of cancer growth rate increasing by enhancement of dose from 6 to 9 J/cm^2 as found by Dobrynin *et al.*, [18]. Table 2 shows that the inhibition of tumor grows percentage is decreasing by 71.9 % for the dose of 6 J / Cm^2 and by 90.1 for 9 J/ cm^2.

TABLE 2 . The effect of PCAP on tumor volume decrement.

Index	**Control**	**G1** Dose =6 J/cm^2	**G2** J/cm^2 9 =Dose
Mean Tumor volume .(mm^3) before treatment	929.5±100	788±38.5	805±71
Mean Tumor volume .(mm^3) after treatment time	3116±406	748±41	273±26.45
Inhibition of Tumor %Growth	-	71.9%	90.1%

Improvement of survival

Figure 6, Shows the variation of the survival percentage versus of the incubation period of tumor implantation for all groups. The results show that the curve of control group exhibit no animal survival not longer than 24 days but mice exposed to plasma doses of both 6 and 9 J/cm^2 survived for a period of 36 day.

This indicates that there is positive effect of cold plasma jet on both tumor growth and survival period of the experimental animals [5,19].

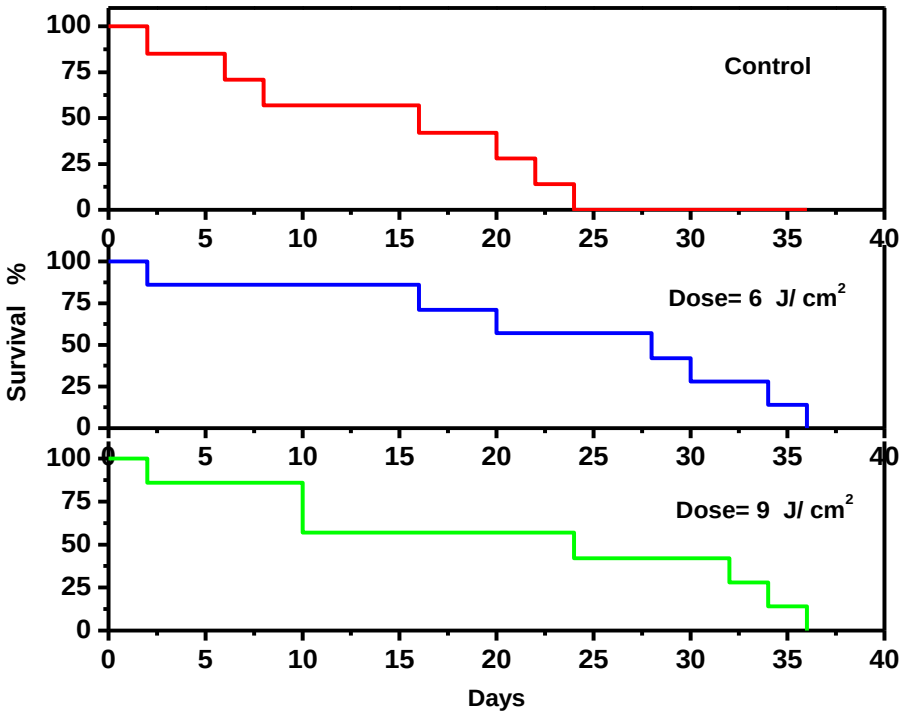

Fig. 6. Effect of PCAPJ on the mice survival .

Discussion

Sever effect of cold plasma treatment on cancerous tissue *in vivo* have been observed. Safety plasma treatment for the whole mouse organism and the absence of skin damages are recorded whereas the thermal effects associated with cold plasma are negligible as the results represented in Fig. 4.

There are many external factors that can induce apoptosis such as short-living reactive chemicals e.g. nitric oxide (NO), reactive oxygen species (ROS), and UV irradiation according to Stoffels *et al.* [9].

Figure 3, indicates clear intensity level of highly reactive radicals such as OH and O which playing an important role in damage of cancer cells. One of the mechanisms of apoptosis induction is the DNA damage which is confirmed practically by histopathological investigations. The damage can also be induced due to formation of hydrogen peroxide (H_2O_2). A hydrogen peroxide molecule, may pass through cell membrane and causes lethal effects such as fatal damage for DNA. This was found according to the results obtained by Imlay *et al.,* [20].

In the Ehrlich tumor model, plasma treatment shows a significant reduction of tumor volume at the end of the treatment period, as compared to control.

These data are in agreement with those obtained by Keidar *et al.*, Keidar *et al.* and walk *et al.* [5, 20, 21].

Finally PCAP shows an increase of 46% of mice life span, and increase in survival period of the animals which reflects a high cytotoxic effect for tumor when it is treated with cold plasma.

Conclusion

- The present study demonstrates a marked antitumor effect of plasma treatment for Ehrlich carcinoma with a significant decrease of tumor volume.
- Plasma treatment lead to enhancement of mice survival interval for treated objects with plasma jet comparing to control group.
- The obtained results indicate that the present therapeutic method may deliver reactive oxygen species (ROS) directly into tumor cells.
- The present results are very promising and highlight the potential of plasma jet treatment as an anticancer inhibitor with little or no toxic side effects.

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تأثير البلازما الباردة على سرطان الإرليك

محمد إسماعيل الجوهرى ، فاروق فهمى الأكشر ، صبحى أحمد غلاب و مصطفى على أبوغزالة
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أدى التقدم الحديث فى بلازما الضغط الجوى إلى تخليق بلازما باردة بدرجة حرارة قريبة من درجة حرارة الغرفة ، وفى الدراسة الحالية تم كشف اثار بلازما الضغط الجوى الباردة على أورام الإرليك المخلقة ، وقد أجريت هذه الدراسة على ثلاث مجموعات من ذكور الجرزان البيضاء الحاملة لورم الإرليك ، كما تم استخدام جهازتوليد البلازما المناسب لمعالجة هذه الخلايا السرطانية. وقد وجد أن الأورام اضمحلت بتعريضها للبلازما الباردة مرة واحدة عبر الجلد. كما تم أيضا دراسة معدل بقاء حياة الجرزان وحجم الورم ، وقد أوضحت النتائج أن ميكانيكية تأثير البلازما الباردة على الخلايا السرطانية هى تأثير أنواع من الأكسجين النشط والذى ينتج مع مسار قتل الخلايا السرطانية وقد أدت هذه الطريقة الى وقف نمو خلايا ورم الإرليك وتعتبر طريقة جديدة ومتطورة لعلاج الخلايا السرطانية مع أقل حدوث للاثار الجانبية .