

The effect of the cold atmospheric plasma on the number of platelets

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Abstract

This study includes the direct influence of (single & multi) dose of Cold Atmospheric Plasma (CAP) on the no. of platelets for mice for different exposure time (15, 30, 60, and 120) sec. the influence of CAP on mice was measured after 1, 2, 3, 7, and 14 day from exposure.

The results obtained in this study indicate that the effect of low doses of CAP on platelets was stimulatory effect in the first few hours from exposure (1day) but the high dose was inhibitory, It was found that after two weeks of exposure that the number of platelets became normal comparable to the control one, and this indicates that plasma effect was removed after this period.

Key words

Cold atmospheric plasma, floating electrode- dielectric barrier discharge, platelets, single dose, multi dose.

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دراسة تأثير بلازما الضغط الجوي الباردة على عدد الصفائح الدموية

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الخلاصة

تشمل هذه الدراسة التأثير المباشر للجرعة (المفردة والمتعددة) من الـ CAP على عدد الصفائح الدموية للفئران لفترة التعرض المختلفة (15, 30, 60, 120) ثانية، حيث تم قياس تأثير CAP على الفئران بعد (1, 2, 3, 7, 14) يوم من التعرض.

تشير النتائج التي تم الحصول عليها في هذه الدراسة إلى أن تأثير الجرعات المنخفضة من الـ CAP على الصفائح الدموية كان تأثيراً تحفيزياً في الساعات الأولى من التعرض (يوماً واحداً) ولكن عند الجرعة العالية كان تثبيطاً، وتبين بعد أسبوعين من التعرض أن عدد الصفائح الدموية أصبح طبيعياً مقارنة بالسيطرة، وهذا يشير إلى إزالة التأثير البلازما بعد هذه الفترة.

Introduction

Cold Atmospheric Plasma (CAP) is an ionized gas generated at near room temperature, in which electrons and heavy particles are in thermal non-equilibrium [1, 2], we can say to Cold Atmospheric Plasma (CAP) is non-thermal because it has electrons at a hotter temperature than the heavy particles that are at room temperature [3, 4], This type of plasma can be no electrical and

thermal damage to the cell surface, absorption the organic material [5, 6] Various methods exist for the production of cold atmospheric plasma such as: Dielectric Barrier Discharge (DBD), Atmospheric plasma jet (APPJ), needle plasma and plasma pencil [3, 6]. Several different gases can be used to produce CAP such as Helium, Argon, Nitrogen, Heliox (a mix of helium and oxygen), and air [3, 7].

CAP had been used in many fields includes: sterilization of infected tissue, inactivation of microorganisms, wound healing, skin regeneration [8], blood coagulation [9-12], tooth bleaching [10, 12-17], and cancer therapy [18, 19].

Many of CAP systems: floating - electrode dielectric barrier discharge and plasma torch Proved the possibility of accelerating the process of blood clotting in vivo and in vitro without any damage to surrounding tissues [20], another study referred to the importance of CAP for clinical treatment because it stimulates platelets in the blood [21].

Because blood is one of the most important tissues of the living body, which can be affected by cold atmospheric plasma, and the importance of platelets in the process of coagulation so the current study, aims to study the effect of cold atmospheric plasma on the platelets.

Materials and methods

A group of mice consist of 250 Balb C male mice (2-3 month) age were purchased from national center for drug control and research/ Ministry of Health / Baghdad, the group of mice divided into 2 groups, one group was exposed to single dose of CAP and the other group was exposed to multiple doses of CAP (the interval time between two doses was 48h) 0, 48 h, 96 h. Hairs of one side of back of every mouse was shaved using hair remover veet. And scraped by blunt scraper so that skin is not injured. The shaved area is about 2 to 3 cm².

The mice were exposed to CAP for different time intervals at 15, 30, 60, and 120 seconds and keep one group untreated as a control. Exposure is performed by using a Lab Made floating electrode–dielectric barrier discharge (FE- DBD) device (which

designed and manufactured in our laboratory). The probe has cylindrical shape with flat base of diameter 2 cm which is smoothly moved over the shaved skin area of mouse back.

Mice are left in different labeled cages for (1, 2, 3, 7, 14) day. Blood which is taken from each Balb C mouse is examined by auto analyzer (Mindray). Finally, Analyses data which obtained using a statistical program (GRAPHPAD).

The method of work

Single dose

To study the effect of single dose of the CAP on the platelets of the mice, five groups of mice each consist of 25 mice were chosen one group was left as control. Other four groups each was exposed to given time of exposure (15, 30, 60, 120) sec.

Each group then ,was subdivided to five group each consist of five mice, the subgroup after exposure were left for different time intervals after exposure (1, 2, 3, 7, 14) day.

The blood of each mice in a give sub group was withdrawal and the test was done.

Multi dose

Study the effect of multi doses, 3 doses were given to the mice four doses were tested (15, 30, 60, 120) sec.

The effects of the doses on the platelets number were measured after (1, 2, 3, 7, 14) days.

Results and discussion

The number of platelets in blood sample was recorded after different intervals from the moment of exposure (1, 2, 3, 7, and 14) day

1-single dose

Table 1 represent the effect of single dose of CAP on the platelets for the different exposure time (15, 30, 60

and 120) sec and after different intervals time (1, 2, 3, 7 and 14) day.

Fig. 1 (a, b, c, d and e) represent the effect of single dose of CAP on

the platelets as function of exposure time, while Fig. 2 (a, b, c and d) represent the same effect but as function of sampling day.

Table 1: Effect of single dose of cold plasma on platelets.

Count no.*10 ⁵ /mm ²										
Intervals time										
Exposure time	1day		2day		3day		7day		14day	
	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD
control	4.5	0.2	4.5	0.2	4.5	0.2	4.5	0.2	4.5	0.2
15 s	6.1	0.8	2.3	0.3	6.2	0.8	4.9	0.9	4.4	0.6
30 s	14.1	0.9	2.6	0.5	7.0	1.0	4.5	0.7	5.2	0.9
60 s	9.4	1.0	6.3	0.6	7.4	0.8	4.7	0.4	4.2	0.3
120 s	4.5	0.8	5.6	0.1	5.2	0.8	5.2	1.1	11.0	0.5

The effect of single-dose of CAP at the low doses (time of exposure 15 sec) on platelets begins after 1day from exposure where it increases compared to control group with percentage 35 % and it was significant (p -value=0.027) as shown in figure (1.a), while after 2 day, the no. of platelets decreases to half with percentage 48 % (p -value=0.00049) as shown in Fig. 1(b). And when the period after exposure was increased to 3 day, the no. of platelets increase with percentage 37 % compared to the control group (p -value=0.023) as shown in Fig. 1(c).

While after 7 and 14 day from exposure as shown in Fig.1(d,e), the no. of platelets almost not effected by CAP at the 15 sec, there was not significant difference.

When the exposure time is increased to 30 sec one can notice that the no. of platelets after 1day increases significantly with percentage 200 % compared to the control group with significant

(p -value =0.0001) as shown in Fig.1(a) but after 2day from exposure it was found that the no. of platelets decreases with percentage 42 % (p -value=0.0033) Fig.1(b) then back to increases after 3 day with percentage 55 % (p -value=0.0119) as shown in Fig.1(c), and after 7 and 14 day, the no. of platelets approach the control group as shown in Fig.1 (d and e).

On the other hand, the maximum value of the no. of platelets was at the time of exposure 30 sec after 1day from exposure, where the increase was with percentage 200 % compared to the control group, with a significant difference ($p < 0.0001$) as shown in Fig.1(a) and 2(b), while the minimum value of platelets was at the time of exposure 15 sec after 2 day, where the decreases with percentage 48 % compared to the control group with significant difference ($p < 0.001$) as show in Fig.1(b) and 2(a).

As it noted from the Table 1 and Fig .1(e) that the effect of single doses

of CAP on the platelets for long period of exposure be slight or non-existent for exposure time (15-60) sec, but at 120 sec, the no. of platelets

increases with percentage 150% compared to the control group, with significant difference ($p < 0.0001$).

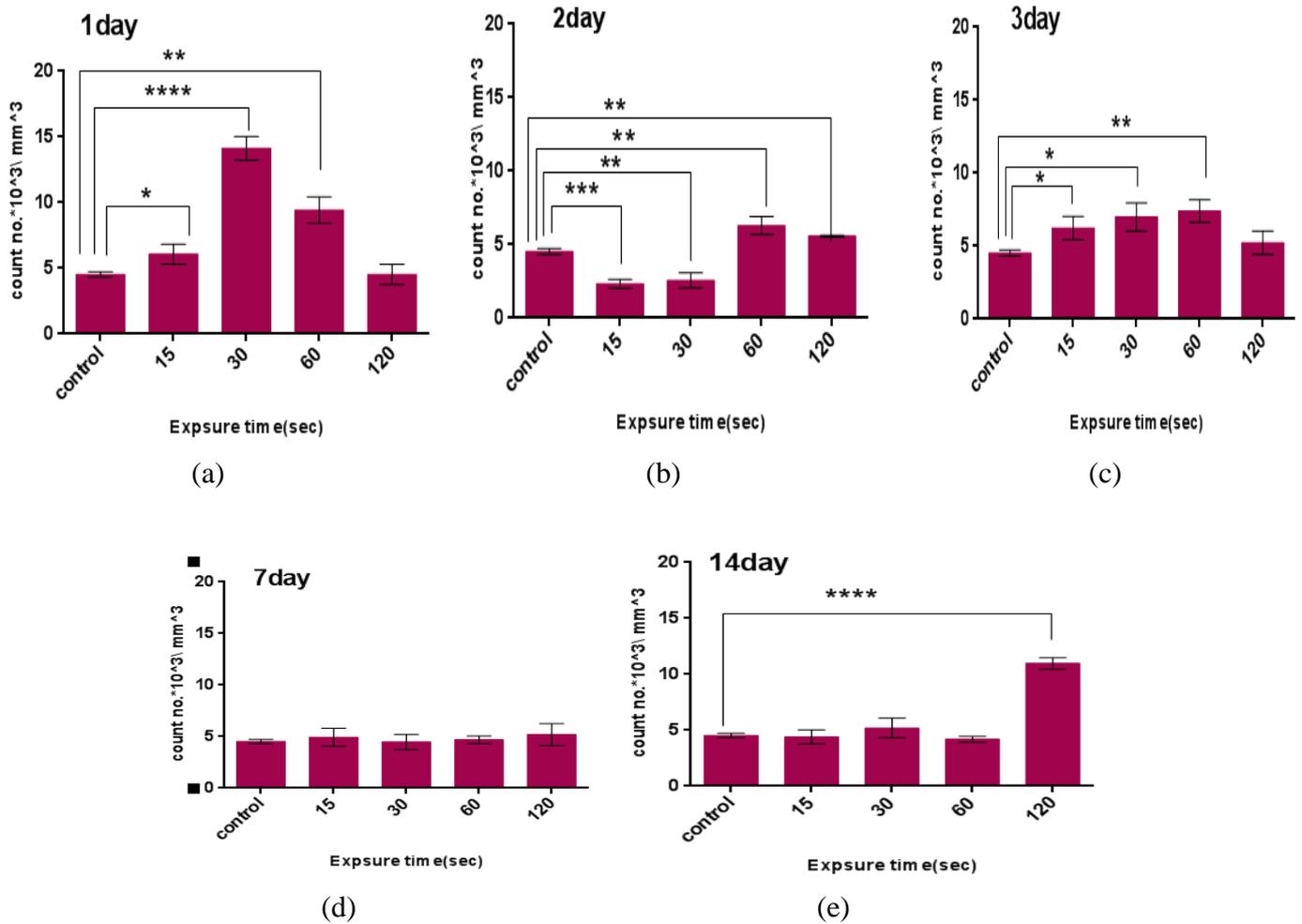


Fig. 1: Effect of number of platelets as function of exposure time (15, 30, 60, 120) sec with stabilized time measured after exposure (a):1day, (b):2day, (c):3day, (d):7day, (e):14day, at the single dose of CAP .Data are expressed as means+SD,* $p < 0.05$, ** $p < 0.01$, * $p < 0.001$, **** $p < 0.0001$ (*) compared with control group.**

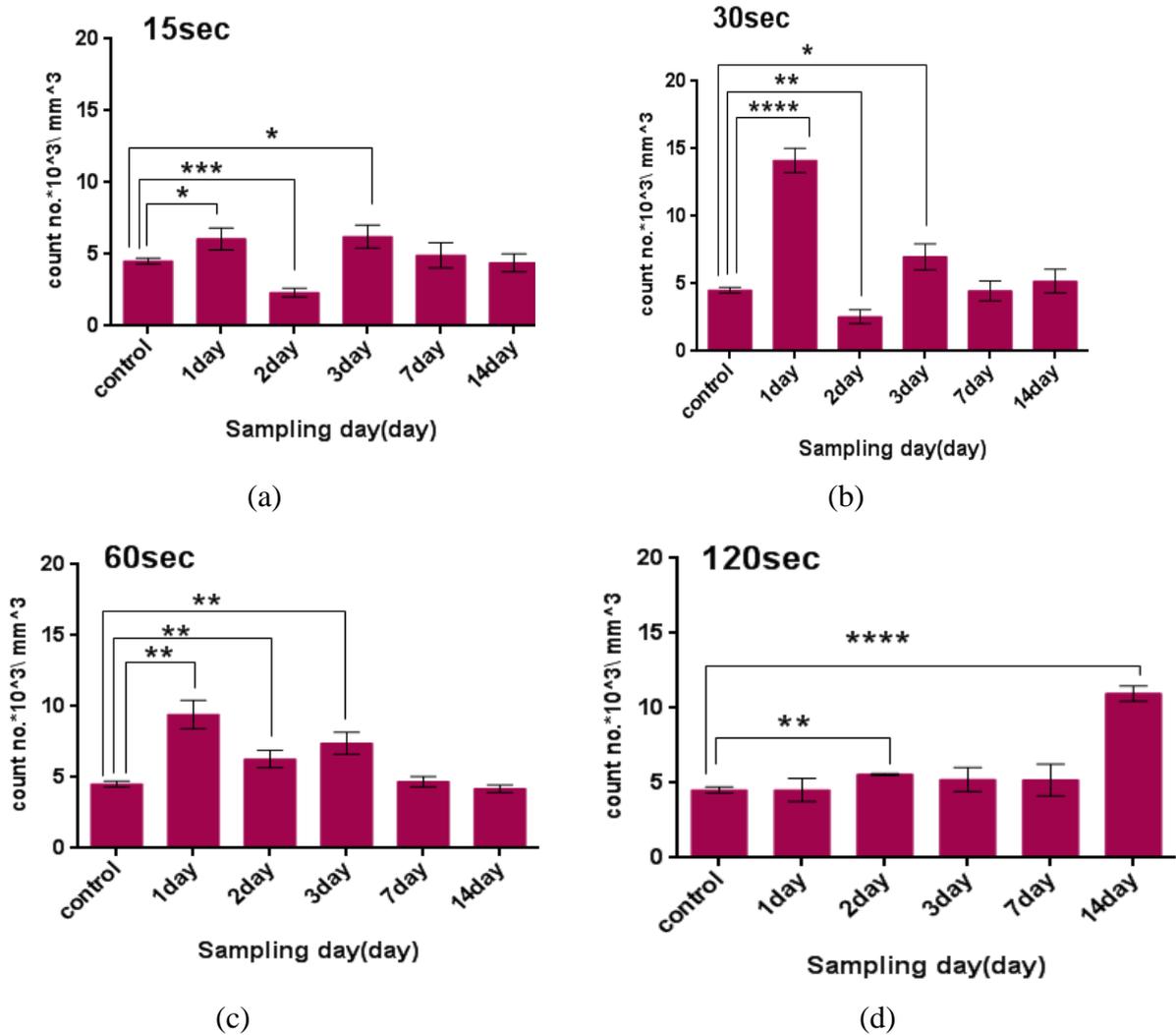


Fig.2: Effect of number of the platelets as function of sampling time (1, 2, 3, 7, 14) day, with stabilized dose of plasma (a):15sec, (b):30sec, (c):60sec, (d):120 sec, at the single dose of CAP. Data are expressed as means+SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (*) compared with control group.

2-multi dose

Table 2 represent the effect of multi dose of CAP on the platelets at the different time (15, 30, 60, and 120) sec and after different intervals time (1, 2, 3, 7, and 14) day, Fig. 3 (a,

b, c, d and e) represent the effect of multi dose of CAP on the platelets as function exposure time, and Fig. 4(a, b, c and d) represent the same effect as function sampling day.

Table 2: Effect of multi dose of cold plasma on platelets.

Count no.*10 ⁵ /mm ²										
Intervals time										
Exposure time	1day		2day		3day		7day		14day	
	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD
control	3.6	0.2	3.6	0.2	3.6	0.2	3.6	0.2	3.6	0.2
15 s	4.2	1.1	3.6	1.0	5.5	1.0	5.8	0.9	4.4	0.9
30 s	7.9	0.7	6.3	0.8	8.0	0.9	5.5	0.9	6.7	1.0
60 s	5.0	0.6	9.2	0.5	8.3	0.7	5.1	0.8	5.1	0.6
120 s	1.6	0.4	4.8	0.5	7.4	0.5	4.9	0.6	4.9	0.9

From Table 2 one can notice that the effect of multi dose of CAP on platelets after 1, 2, 3, 7, 14 day from exposure at the low doses (15, 30, 60) sec was stimulatory but it become inhibitory at the high doses (120) sec.

Also we noted the maximum value of platelets at the 60 sec after 2 day from exposure where the no. of platelets increases with percentage 155% with significant difference ($p < 0.0001$) as shown in Fig.1(b) and 2(c), but the minimum reading of platelets in 120 sec after 1day of exposure, the no. of platelets decreases with percentage 55% with significant difference ($p < 0.01$) as shown in Fig.1(a) and 2(d).

The effect of multi dose of CAP on the platelets at the long time (14) day

was stimulatory at the all doses except at the 120 sec the effect was inhibitory. From this obtained results can conclude that the effect of the cumulative dose of CAP was similar to the effect of single dose.

CAP is an important tool for medical application, especially in the treatment of cancer such as breast cancer [22] and the ability of CAP to rapidly the process of clotting, it is a promising and important in the surgical operations to control bleeding cases [20]. From the above results we note that cold plasma has the ability to increase platelets and thus help to coagulation of blood.

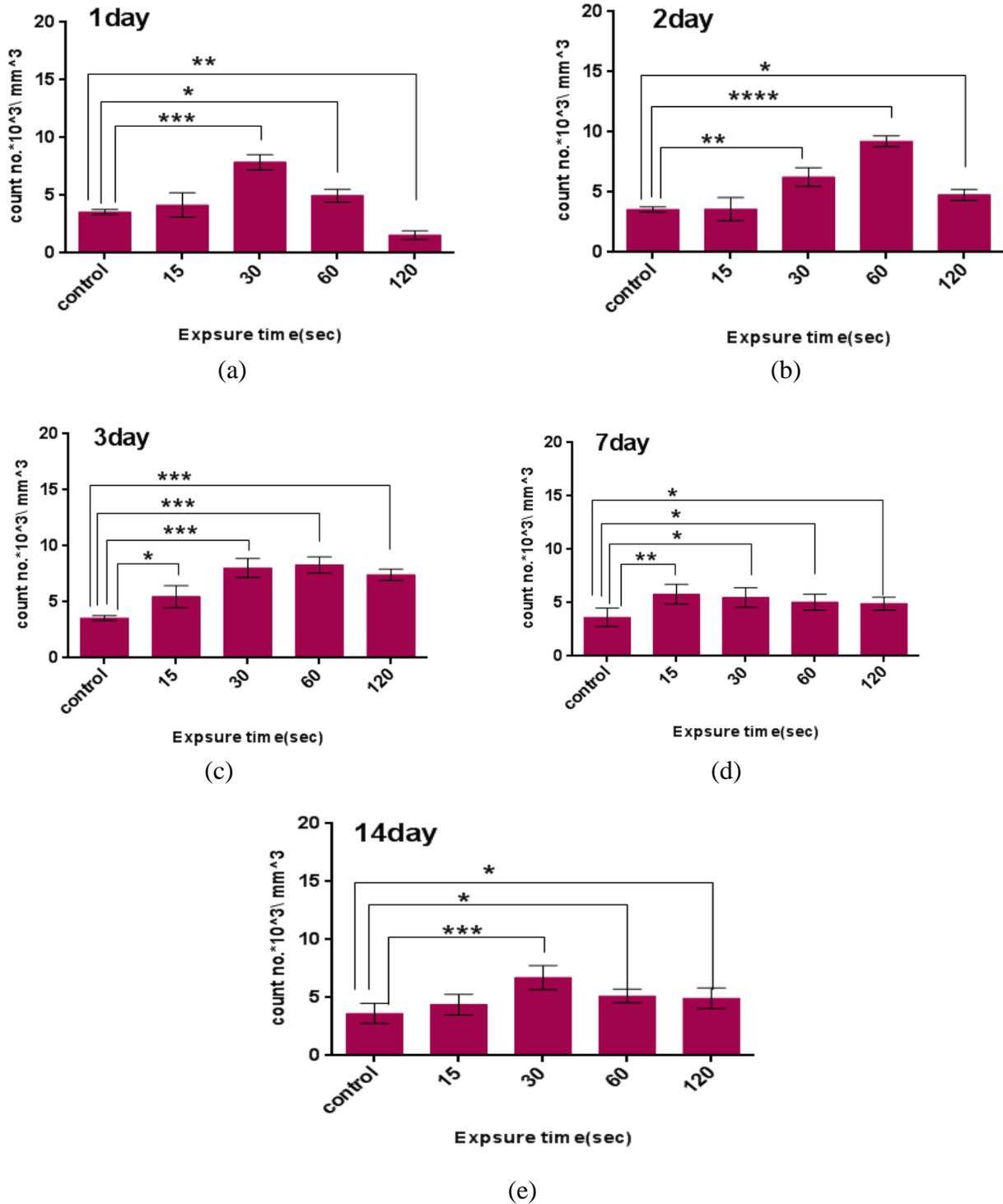


Fig.3: Effect the number of platelets as function of exposure time (15, 30, 60, 120)sec with stabilized time measured after exposure (a):1day, (b):2day, (c):3day, (d):7day, (e):14day, at the multi dose of CAP .Data are expressed as means+SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (*) compared with control group.

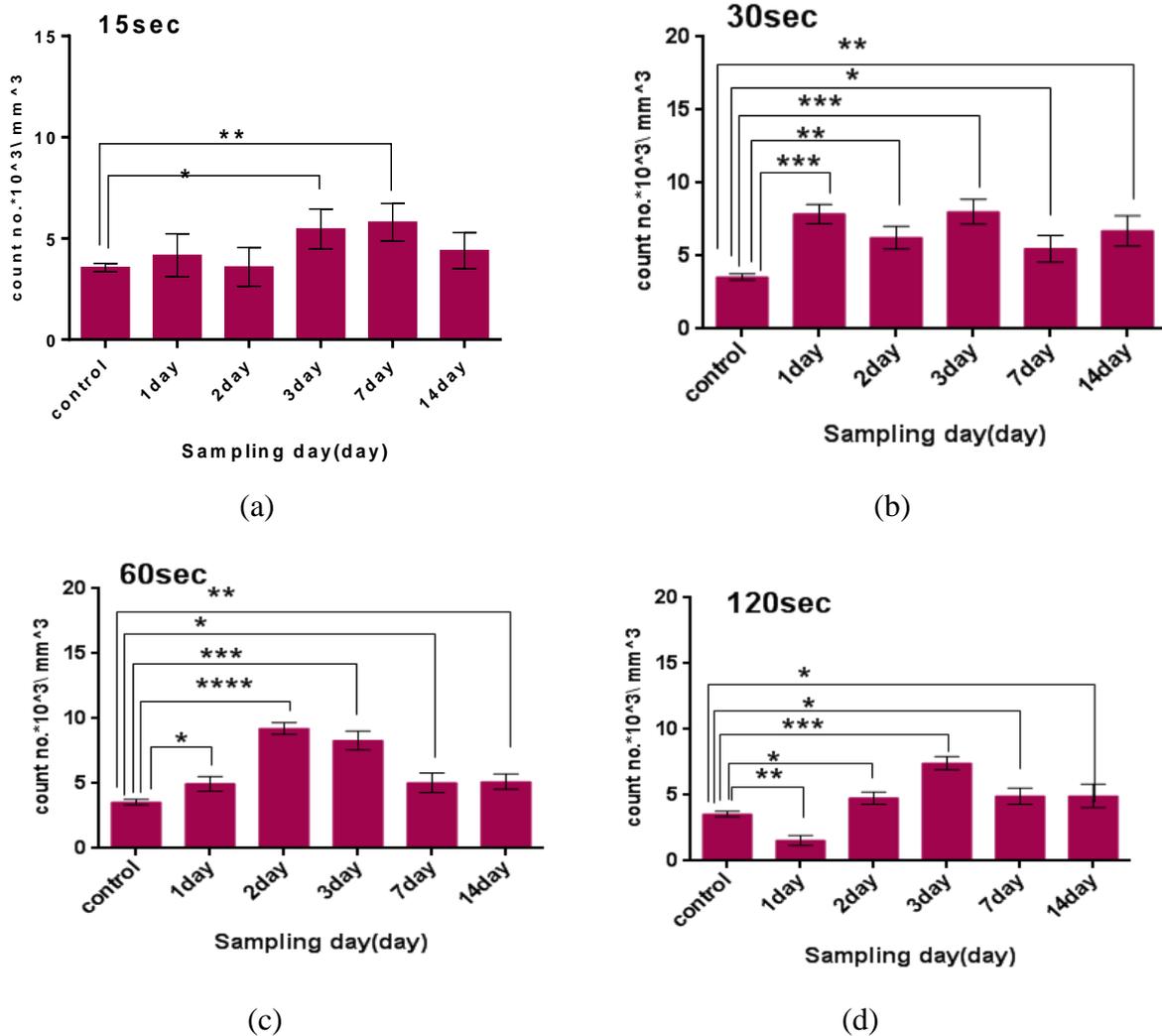


Fig.4: Effect the number of the platelets as function of sampling time after exposure (1, 2, 3, 7, 14) day, with stabilized dose of plasma (a):15sec, (b):30sec, (c):60sec, (d):120sec, at the multi dose of CAP. Data are expressed as means+SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (*) compared with control group.

Conclusion

In our study, the number of platelets was changed when laboratory mice were exposed to one or multiple doses of CAP compared to control.

Also it notices that there are statistically significant differences between the control group and exposure groups to CAP with different time.

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