Plant Archives Vol. 20, Supplement 1, 2020 pp. 526-531 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STUDY OF THE EFFECT OF COLD PLASMA ON THE REPRODUCTIVE ENDOCRINOLOGY OF MALE RATS

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Abstract

Cold atmospheric plasma (CAP) is used widely in medical and biological fields because of non-thermal effected. Direct application of plasma is preferred in medical functions, so, direct application of cold plasma has obtained by the floating electrode dielectric barrier discharge (FE-DBD) system. The purpose of this paper to review the effect of (CAP) on the reproductive hormones (testosterone, LH, E2, progesterone, for male rats. The study appeared that no significant effect on E2 and progesterone hormone for all time of exposure, besides this significant difference in LH hormone ($P<0.05$) at 15 sec, ($P<0.0001$) at 30, 90 sec and ($P<0.001$) at 60 sec of exposure to plasma. Added to that significant difference ($P<0.01$) at 15, 30, 60 sec and no significant differences at 90 sec for testosterone hormone.

Keyword: FE-DBD, CAP, LH, E2, Progesterone, testosterone.

Introduction

Plasma is a quasi-neutral gas which exhibited a collective behavior (Chen, 1984). It is consist of different species positive and negative ions, neutrals, reactive species, metastable, free radicals and photons (Weltmann, Metelmann, & von Woedtke, 2016).

Plasma is classified into two main groups according to its temperature: high temperature (fusion plasma) and low temperature plasma (gas discharge). Besides, low temperature plasma is divided into two groups thermal and non-thermal plasma, when the temperature effected is dependent essentially in treatment is called thermal plasma, and when the constituents of plasma are used is called non-thermal plasma (Vijay Nehra).

Cold plasma is preferred in medical and biological application as a result to its temperature (< 40°C), it is usually equal to room temperature, it can be exposed to biological tissues without any thermal damage (Fridman *et al.*, 2008), and it is selective in its treatment (Dobrynin, Fridman, Friedman, & Fridman, 2009).

These enable many new medical and biological applications, including sterilization surfaces, living tissues and inactivation of bacteria (Att, Al Haideri, & Murbat, 2019; Ayan *et al.*, 2009; Cooper *et al.*, 2009; Fridman *et al.*, 2008; Joshi *et al.*, 2010; Vaze *et al.*, 2010), blood coagulation (Ayan *et al.*, 2009; Kalghatgi *et al.*, 2007), wound healing(Naderi & Zaefizadeh, 2017), treatment for various types of scars and skin conditions (Shimizu & Ikehara, 2017), dentistry (Wang, Mi, Li, Jin, & Dong, 2017), cancer treatment (Chen, Cheng, Lin, & Keidar, 2016; Metelmann *et al.*, 2018) and among others (Tanaka *et al.*, 2017). There are many sources (Vijay Nehra) of cold atmospheric plasma such as dielectric barrier discharge (Liao *et al.*, 2018; Rad & Davani, 2016; Tran & Harada, 2018), plasma jet (Schmidt *et al.*, 2017) , and corona discharge (Arola, Kallioinen, Reinikainen, Hatakka, & Mänttäri, 2018).

Non-thermal plasma that is generated by dielectric barrier discharge (DBD) at atmospheric pressure in gas when a high voltage of short duration pulses or time-varying waveform is applied between two electrodes. The generated plasma is safe electrically without substantial gas heating, for this reason, it is preferred in medical and biological application.

Floating electrode dielectric barrier discharge technique (FE-DBD), is a special kind of DBD, it can be used to get direct application with the desired target, the desired target represented the second electrode.

When the living tissues are exposed to cold plasma caused many reaction leads to change in the liquid environment of cells, different biological reactive species are generated (ROS, RNS) (Weltmann *et al.*, 2016).

Fertility hormones are secreted from different endocrine glands in the body, such as testosterone or androgens, it is a steroid hormone naturally androgen secreted. It is mainly produced by the Leyding cells located in the testes. It is responsible for the development of secondary sex characteristics, such as the accessory sex organs, the prostate, seminal vesicles and the growth of facial, pubic and auxiliary hair.

Estrogen hormone (E2) is produced essentially by the ovary, placenta, in smaller amount by the adrenal cortex, and the male testes. It is secreted into blood stream where bound to sex hormone binding globulin (SHBG).

Progesterone is a steroid hormone is synthesized from both tissue and circulating cholesterol. The mainly production are the adrenals, ovaries and the placenta during pregnancy. The primary role of progesterone is release by the reproductive organs. In male, progesterone is a necessary for the production of corticosteroids and androgens.

LH hormone in male stimulates Leydig cells to produce testosterone. Testosterone provides negative feedback to regulate LH secretion at the level of the anterior pituitary gland and hypothalamus (Medical & Kaplan, 2017).

The aim of this study to evaluate the effective of cold plasma on fertility hormones for male rats.

Material and Method

Experimental Animals.

(125) healthy adult male rats Albino/bulb C were used for the experiment, there are purchased from the animal house of Iraqi Center for Cancer and Medical Genetic Research. The aged between (2-3) months, the average

weight about 250±3.194g. there are divided into many groups, control group without plasma exposure and treated groups according to time exposure to CAP (15, 30, 60, and 90 second), each group consist of 30 rats. Then, each group subdivided to six groups (n=5) according to time interval after exposure to CAP (1, 2, 3, 7, 14, 28) days.

Floating Electrode Dielectric Barrier Discharge (FE-DBD) System

(FE-DBD) system is a special kind of DBD, it is consist from two electrode but one of them is floating. The system is designed and made up by our team for production non thermal plasma, it is produced safely plasma, it is non damaging system with low current discharge. Mainly electrode is made up from stainless steel rod that covered by Teflon and barrier from the live body by quartz, it is safely contact with the treatment target, it is connected to high voltage source of the power supply about 23 kV with little current about 114 µA , while the second electrode is an organism or any tissue surface that want to exposed to plasma.

Methodology

Preparing the laboratory animals by removing hair from the dorsal side to ensure direct contact of the CAP with the skin, exposed the grouped to CAP for different time (15, 30, 60, 90 sec) except the control group lift without plasma exposure.

A volume of 5 ml of blood samples was collected directly from heart of animals by heart puncture into Gel tube for extracting the serum by centrifuged at a speed of 2500 rpm for 10 minutes. The supernatant serum is collected by micro pipit, it is clear, and non- hemolytic. Then it was divided into fifth parts and stored individually in eppendorf at (- 20 ^{o}C) for measurement the hormones by (ELISA) technique.

Hormones Concentration Assay

The concentration of LH, Estrogen, progesterone and Testosterone hormones are measured by Enzyme Linked Immunosorbent Assay (ELISA) technique micro plate reader (Awareness USA) according to instruction of (Rapid Labs Limited, UK).

Statistical Analysis

It is implemented using one way (ANOVA) analysis of variance to verify the least significant difference (LSD) between groups and t-test analysis of variance to verify (LSD) between controls with each group separately using (Graph Pad Prism 7.04) software.

Results and Discussion

The effected of CAP on fertility hormones are different from each others, the results of LH hormone show that significant change ($P<0.05$) for the first group (15 sec) and ($p<0.001$) for the group of (60sec) and significant difference ($p<0.0001$) for (30, 90 sec) as shown in tables (1) and figures (4.8) between all days of the group compared by ANOVA test, when compare the plasma treated group with control group by t-test, for the first group (15 sec), there are no significant differences from day 1, 7 to day 28, and there are significant difference at the second and third day ($P<0.05$) as shown in figures (1-a).

For the second group (30 sec), there are no significant differences between plasma-treated group and the control group from day 1, 2 and day 7, and there are significant difference for the day 3, 14 days ($p<0.01$) until day 28 ($P<0.05$) as shown in figures (1-b). For the group of (60 sec), there are significant difference from the second day ($p<0.01$) until the first week ($p<0.001$), then from the second week there are no significant difference, and the group (90 sec) there are significant difference between plasma-treated group and the control group for all interval groups unless the second day the effected no significant as shown in figures (1-c,d).

When the animal is exposed to insufficient secretion of LH, it is caused weakness in the production of sperm, so, CAP effected is positive on the LH hormone. The concentration of LH is about control value or significant little increased.

Table 1 : Effect of CAP on the LH (mIU/mL) concentration.

Exposure time	1 day Mean ±SD	2 day Mean ±SD	3 day Mean ±SD	7 day Mean ±SD	14 day Mean ±SD	28 day Mean ±SD
control	4.48±0.024	4.48±0.024	4.48±0.024	4.48±0.024	4.48±0.024	4.48±0.024
15 s	4.595±0.19	4.766±0.038	4.721±0.042	4.612±0.125	4.563±0.134	4.629±0.087
30 s	4.512±0.058	4.489±0.013	4.782±0.037	4.680±0.164	4.701±0.007	4.709±0.045
60 s	4.475±0.022	4.762±0.044	4.673±0.007	4.770±0.019	4.652±0.156	4.591±0.175
90 s	4.673±0.014	4.600±0.110	4.681±0.000	4.685±0.042	4.738±0.073	4.658±0.043

Table 2 : Effect of CAP on the E2(pg/mL) concentration.

Exposure time	1 day Mean ±SD	2 day Mean ±SD	3 day Mean ±SD	7 day Mean ±SD	14 day Mean ±SD	28 day Mean ±SD
control	56.379±0.313	56.379±0.313	56.379±0.31	56.379±0.31	56.379±0.313	56.379±0.31
15 sec	56.501±0.422	56.837±0.447	56.582±0.34	56.654±0.51	56.591±0.448	56.667±0.15
30 sec	56.415±0.539	56.810±0.402	56.683±0.15	56.883±0.46	56.472±0.504	56.683±0.15
60 sec	56.486±0.060	56.561±0.070	56.346±0.22	56.487±0.25	56.765±0.297	56.620±0.29
90 sec	56.566±0.213	56.420±0.110	56.521±0.33	56.647±0.36	56.710±0.386	56.699±0.30

Table 3 : Effect of CAP on the progesterone (ng/mL) concentration.

Exposure time	1 day Mean ±SD	2 day Mean ±SD	3 day Mean ±SD	7 day Mean ±SD	14 day Mean ±SD	28 day Mean ±SD
control	0.560±0.39	0.560±0.39	0.560±0.39	0.560±0.39	0.560±0.39	0.560±0.39
15 s	0.320±0.09	0.369±0.13	0.401±0.08	0.366±0.12	0.390±0.08	0.369±0.02
30 s	0.311±0.08	0.374±0.30	0.416±0.11	0.472±0.14	0.542±0.20	0.515±0.11
60 s	0.415±0.09	0.496±0.17	0.471±0.08	0.442±0.09	0.494±0.10	0.544±0.02
90 s	0.460±0.11	0.475±0.12	0.481±0.06	0.464±0.07	0.452±0.11	0.448±0.11

Table 4 : Effect of CAP on the Testosterone (ng/mL) concentration.

Exposure time	1 day Mean ±SD	2 day Mean ±SD	3 day Mean ±SD	7 day Mean ±SD	14 day Mean ±SD	28 day Mean ±SD
control	1.368±0.112	1.368±0.112	1.368±0.112	1.368±0.112	1.368±0.112	1.368±0.112
15 sec	1.333±0.024	1.461±0.027	1.489±0.009	1.331±0.017	1.200±0.109	1.275±0.080
30 sec	1.117±0.253	1.157±0.148	1.062±0.069	0.869±0.019	1.030±0.331	1.414±0.246
60 sec	1.181±0.095	0.843±0.339	0.882±0.169	1.444±0.343	1.249±0.138	1.284±0.115
90 sec	1.158±0.193	1.061±0.051	1.014±0.097	1.339±0.214	1.146±0.255	1.194±0.219

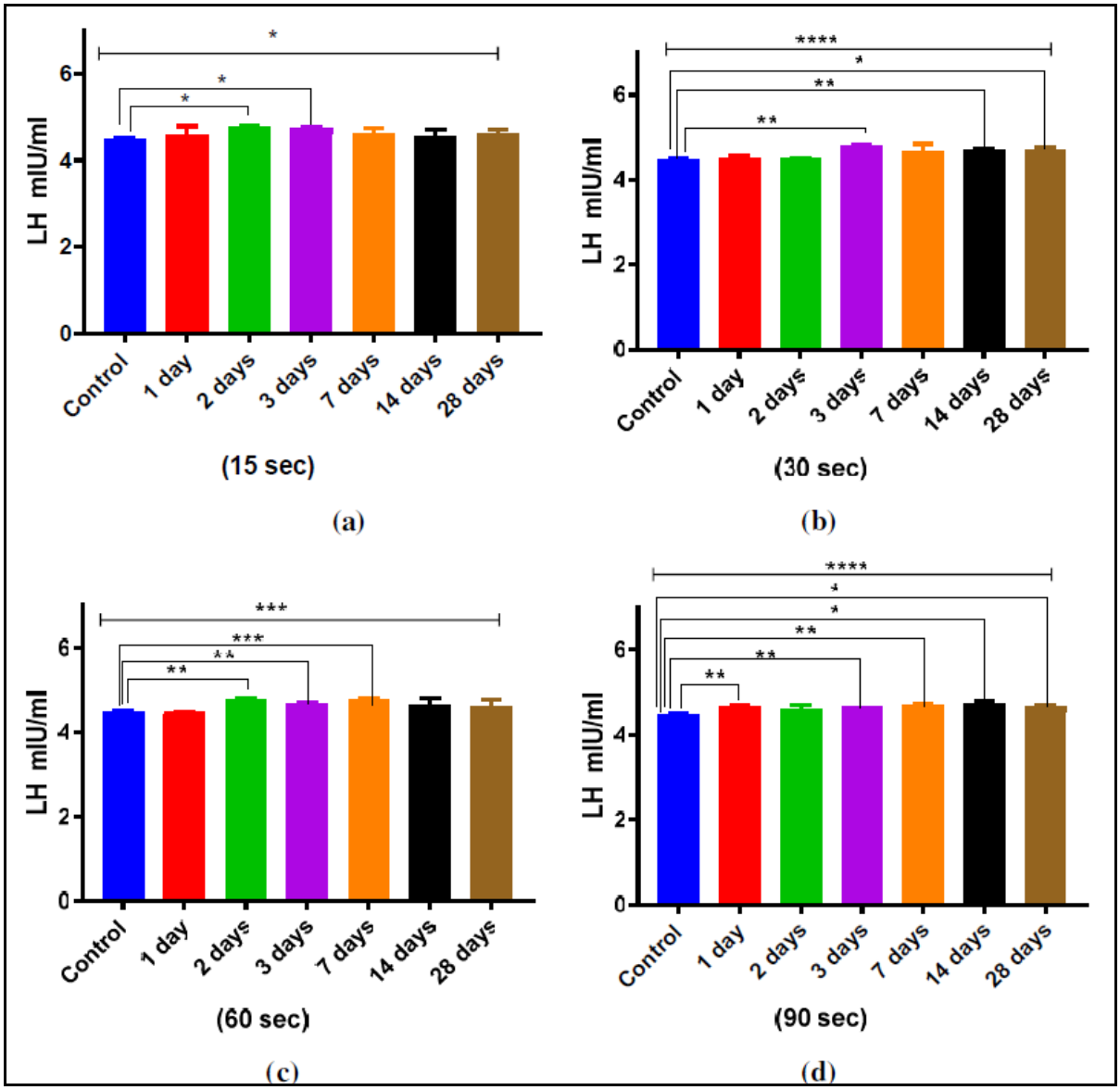

Fig. 1 : Effect of CAP on the LH concentration with time after exposure (1, 2, 3, 7, 14, and 28) days for different time exposure to CAP (a) 15 sec. (b) 30 sec. (c) 60 sec. (d) 90 sec. Data are expressed as means ±SD,*p<0.05, **p<0 .01, ***p<0 .001, **** p<0.0001 (*) compared with control group.

There are no significant difference on the Estrogen and progesterone hormones for male rats between all plasma-treated groups and the control group for all time of exposure (15, 30, 60, and 90 sec) as shown in table and figure (2,3).

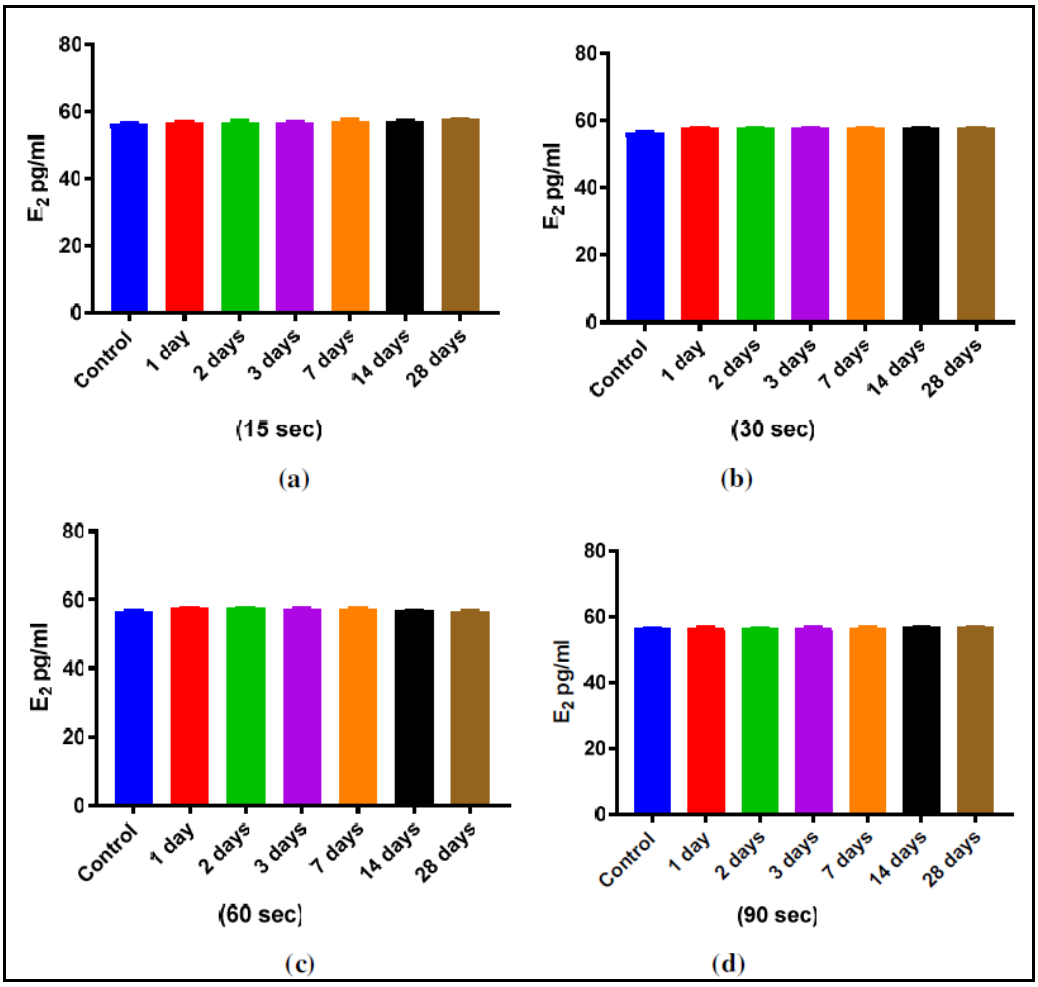

Fig. 2 : Effect of CAP on the Estradiol concentration hormone with time after exposure (1, 2, 3, 7, 14, and 28) days for different time exposure to CAP (a) 15 sec. (b) 30 sec. (c) 60 sec. (d) 90 sec.

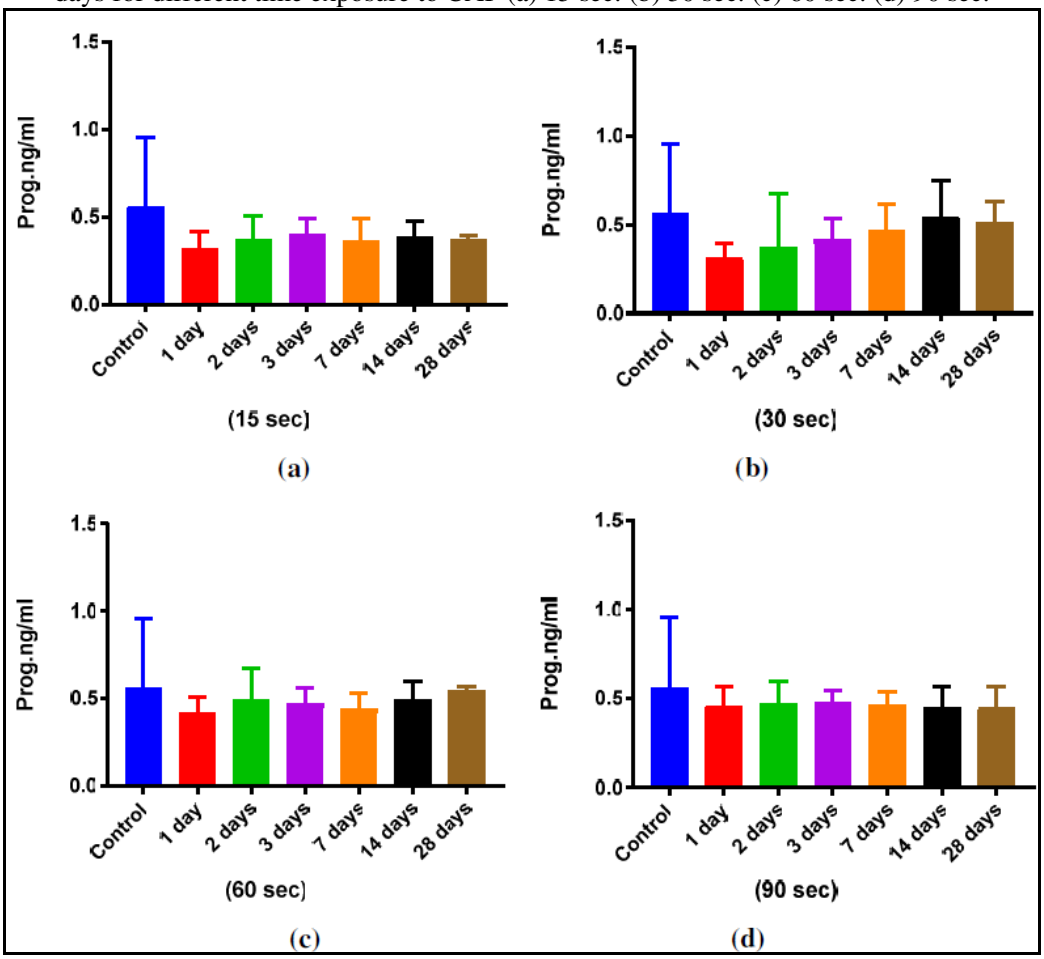

Fig. 3 : Effect of CAP on the progesterone concentration hormone with time after exposure (1, 2, 3, 7, 14, and 28) days for different time exposure to CAP (a) 15 sec. (b) 30 sec. (c) 60 sec. (d) 90 sec. Data are expressed as means ±SD.

Testosterone hormone had a significant difference (P<0.01) when compared all plasma-treated groups for all exposure time unless the group of (90sec) with the control group. While the concentration of testosterone hormone in the serum for male rats had no significant difference when compared each treated group with control group for time of exposure (15, 30, 60, 90 sec), unless day 3 to day 7 for the time (30 sec) significant difference (P<0.01) and day 2 at time 60 sec there are significant difference (P<0.05) as shown in table and figure (4). our result agreement with the previous done by J.J. Zhang (Zhang *et al.*, 2018), there are shown that no significant effected of CAP on the Estrogen and progesterone hormones and significant effected (P<0.01) on the testosterone hormone for the chickens.

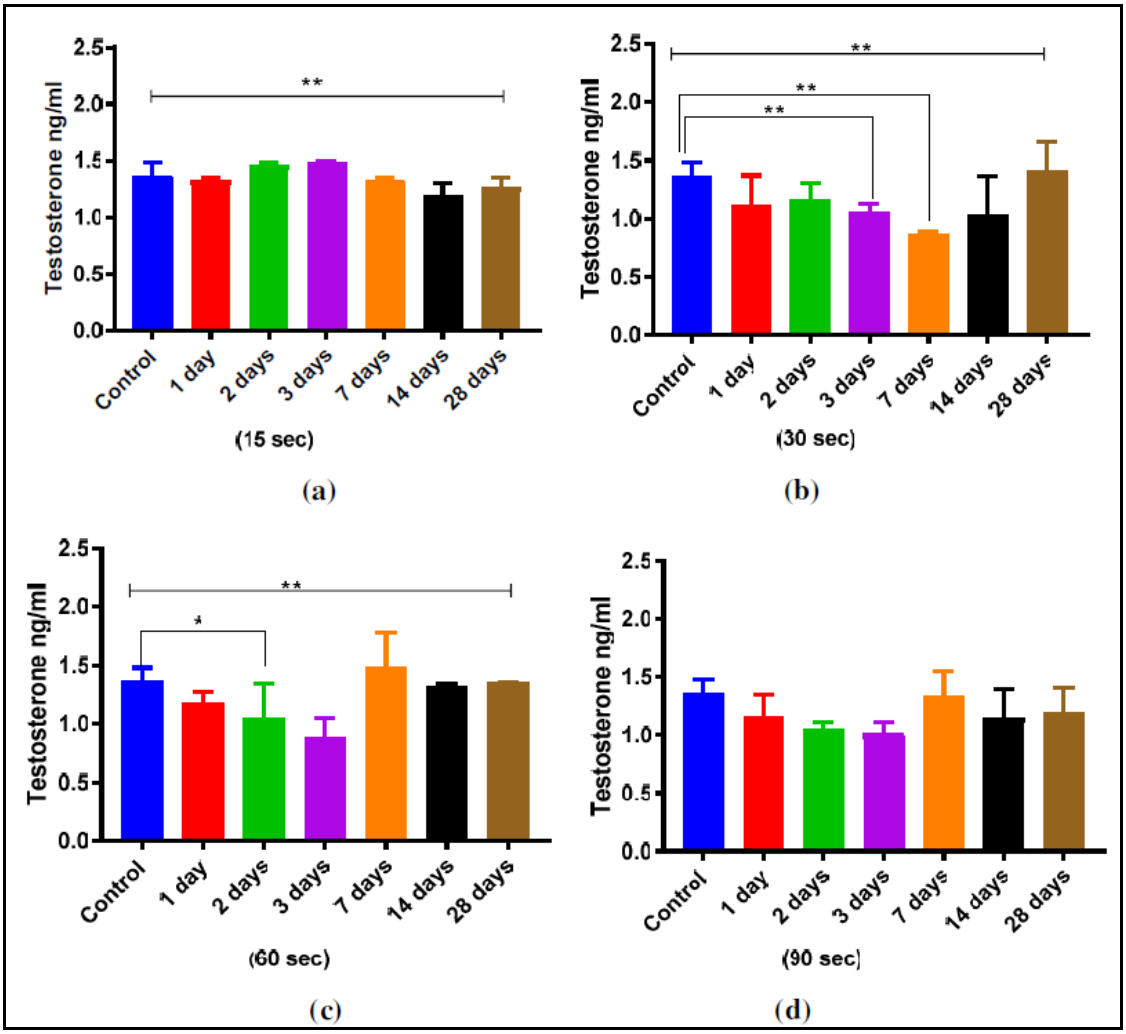

Fig. 4 : Effect of CAP on the Testosterone concentration with time after exposure (1, 2, 3, 7, 14, and 28) days for different time exposure to CAP (a) 15 sec. (b) 30 sec. (c) 60 sec. (d) 90 sec. Data are expressed as means ±SD,*p<0.05, **p<0 .01, ***p<0 .001, **** p<0.0001 (*) compared with control group.

Conclusion

In the present study non thermal plasma was obtained from FE-DBD and applied directly on the libratory animals (male rats) for different time of exposure (15, 30, 60, and 90 sec) and show the effected after (1, 2, 3, 7, 14, 28 days), the CAP have significant effected on the LH hormone for all time of exposure (15, 30, 60, and 90 sec), and significant effected on the testosterone hormone for all time of exposure unless (90 sec) there are no significant. While there are no significant difference on the Estrogen and progesterone hormones for all time of exposure.

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