The Effect of cold microwave plasma on Hormones and Living tissues of mouse females Using Digital Image Processing

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Abstract. The microwave plasma now been used in medical application helping to cut without any damage to the health tissue. The microwaves plasma system worked with 2.45 GHz microwaves frequency, 170 Volt applied voltage, 2 ℓ/min argon gas flow rate and 10 mm discharge tube diameter. Eight female albino mice were divided into two groups one control and one treated with microwaves plasma at 60 second. the results were positive and significantly increase in the follicular stimulating hormone (FSH) and the luteinizing hormone (LH) level. Digital image processing and Histopathology of the uterus is applied to search of effect of the microwave plasma on this organ. The segmentation process represented by thresholding is applied as a first step in the pre-processing. The statistical features is used to explained the changes which occur in the Uterus of the mice.

1. Introduction

Ionized gas or what is known as ( Plasma ) is a mix gas for equal numbers of positively charged ions and negative electrons [1]. The plasma can be totally ionized, as the plasma in the Sun, or fractionally ionized as in fluorescent lamps which have a huge number of neutral atoms [2]. We can differentiate two main groups of plasmas Thermodynamic equilibrium: thermal plasma when the(T electron ≈ T ion ≈ T gas) in 10,000 K temperature and non-thermal plasma when the(T electron > T ion ≈ T gas) in (300 -1000) K temperature [3,4]. The non-thermal plasma can produced by microwave, usually with pressure(10^{-5})torr up the atmospheric pressure and continues wave with power ranging amidst several watts and hundreds of kilowatt[5]. Microwave plasma generated in high and low level of absorbed power, large rang of pressure and possibility of sterilized treated a special area in the skin without damaging to surrounding tissue [6,7]. This review explores the latest major achievements in the field, focusing on the biological effects, mechanisms of action, and clinical evidence of CAP applications in areas such as skin disinfection, tissue regeneration, chronic wounds, and cancer treatment and sperm motility[8,9]. Image segmentation is one of the best methods used in image processing. And benefit us image segmentation is to partition the basic image into several parts. It can used as a pre-processing step for another image processing ways. There are many ways for image segmentation methods used for image processing [10]. simplest segmentation process is a thresholding. It can extract the object from the background by grouping the intensity according to the threshold value [11]. Statistical features that are gained of the grey level co-occurrence matrix which is contrast, correlation and energy. The category of second order statistics includes gray level co-occurrence matrix. Haralick et al suggest that there are 14 features that can be obtained from GLCM which contain information about image textural characteristics GLCM has information about the position of pixel with similar grey level values [13].
### 2. Methodology

**Thresholding:**

Thresholding considered as simplest methods used in segmentation process. This method divides the image into one region of all the pixels with a specific threshold and rejects the entire pixel below the suggested threshold. The thresholding algorithms do not use spatial information of an image and they usually fail to segment objects with low contrast or noisy images with varying background [14].

\[
f(x, y) = \begin{cases} 
1 & \text{if } f(x, y) > T \\
0 & \text{if } f(x, y) \leq T
\end{cases}
\]

\( T \) represented the threshold value. \( x, y \) is the coordinates of the threshold value point. \( f(x, y) \) represents the intensity value of the pixel at \( (x, y) \).

**Co-Occurrence Matrices:**

The spatial gray level co-occurrence matrix is used to evaluate the properties that connected to second order statistics which describe the relation between pixels or sets of pixels (usually two). Using the (GLCM) method become one of the most well-known and broadly utilized texture features, depend on the common probability distributions of pairs of pixels, rather than utilizing gray level co-occurrence matrix straight to measure the textures of images. Four features can be obtained from this matrix which can consider for studying the uterus texture of mice [15].

**Second-Order Statistics Features:**

The related texture feature used to study the mice uterus are:

- The (Correlation) is a measures how a pixel is correlated to its neighborhood pixels. The positive image when the value lies (+1) and negatively image when the value lies between (-1 and +1). [16,17].

\[
\text{Correlation} = \frac{\sum_{i,j=0}^{G-1} (p(i,j)-\mu_x \mu_y)}{\sigma_x \sigma_y} \quad \text{...(2)}
\]

\( \mu_x, \mu_y \): mean value in the x and y direction , \( \sigma_x, \sigma_y \): variance of x, y and

- The ( Homogeneity ) that is a give the distribution value of the closes elements of(GLCM) the gray level co-occurrence matrix. Homogeneity gives value between the( 0 and 1) it measure the purity of the tissue[17].

\[
\text{Homogeneity} = \sum_{i,j=0}^{G-1} \frac{p(i,j)}{1+(i-j)^2} \quad \text{...(3)}
\]

- The (Contrast) is a give local variations in the(GLCM and determines the intensity difference between a pixel and its neighborhood [18].

\[
\text{Contrast} = \sum_{i,j=0}^{G-1} (i-j)^2 p(i,j) \quad \text{...(4)}
\]

- The Energy is the sum of squared elements. Its range is from 0 to 1 [18].

\[
E = \sum_{i,j=0}^{G-1} (p(i,j))^2 \quad \text{...(5)}
\]

Where \( p(i,j) \) the probability density of occurrence of the intensity in two dimension.

**Experimental**

Eight female albino mice were taken(28-35) gm, 4 of them control not exposed to microwaves plasma and 4 are exposed to plasma for sixty seconds. Exposure doses were repeated for 3 days to determine the effect of microwave plasma in hormones level and one minute to study the effect of microwave plasma in living tissue on uterus and Hormones . Microwave plasma generated from this system were applied on mice which shows in figure (1) and worked with 2.45 GHz microwaves frequency, 170 Volt applied voltage, 2 ℓ/min argon gas flow rate and 10 mm discharge tube diameter. Blood samples were collected from mice by heart puncture and the serum was separated by centrifuge
(5417R-Chain) Serum FSH, LH levels were determined by using (ELISA) kits (mono bind) and reader of absorbance by ELISA reader (Human, Germany).

3. Results and Discussion

Table (1) shows the results of the follicular stimulating hormone (FSH) and the luteinizing hormone (LH). The results were measured at control (before plasma treatment), when the mice exposed to microwave plasma at 60 sec. The results showed the effect of microwaves plasma on FSH and LH level, the results were The significantly increase (P<0.05) in hormones level follicular stimulating hormone (FSH) and luteinizing hormone (LH).

Figure(2) show the texture Image for the uterus in the control case. Table (2) and figure (4) represent the texture features for the control case.

From the figures (3) and table (3) show the value of the contrast decrease by the time exposure this means that the texture become more regular, while the correlation and the energy degrease with the time exposure.

Figure (5) represent the contrast decrease with exposure time. So is which energy decrease with time exposure. The correlation increase with time exposure.

This means that the texture become more regular and more Homogenous. The tissue become more pure.

4. Conclusions

The microwave plasma technique is show used to effected on the Uterus mice during the exposure to cold plasma. Exposed to microwave plasma cause to increase follicular stimulating hormone and luteinizing hormone. The statistical features and the graphs, show that effect the value of the contrast decrease by the time exposure this means that the texture become more regular, while the correlation and the energy degrease with the time exposure.

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Table (1): Serum concentrations of FSH and LH of mice treated with microwaves plasma, and control groups.

| Groups       | FSH mIU/ml (mean +SD) | LH mIU/ml (mean +SD) |
|--------------|-----------------------|----------------------|
| Control      | A 3.88 ± 0.2129        | A 3.510 ± 0.2990     |
| second60     | B 6.06 ± 0.8622        | B 4.715 ± 0.1852     |
| LSD          | 0.946                 | 0.374                |

Table (2) the statistical features for the control case

|          | Contrast | Correlation | Energy | Threshold |
|----------|----------|-------------|--------|-----------|
| Imag1(Cont) | 0.8275   | 0.8066      | 0.3590 | 120       |
| Image2(Cont) | 0.4892   | 0.7769      | 0.3049 | 120       |
| Image3(Cont) | 0.9444   | 0.8522      | 0.2807 | 145       |
| Image4(Cont) | 0.8192   | 0.7538      | 0.2166 | 125       |
Table (3) The statistical features for the exposure case

| Image     | Contrast | Correlation | Energy | Threshold |
|-----------|----------|-------------|--------|-----------|
| img1(Expos) | 0.6546   | 0.8069      | 0.2166 | 130       |
| Image2(Expos) | 0.4147   | 0.5863      | 0.2785 | 130       |
| Image3(Expos) | 0.5258   | 0.6053      | 0.1661 | 135       |
| Image4(Expos) | 0.6701   | 0.7567      | 0.2097 | 135       |

Figure (1) Microwave plasma applied on the mice
| No. of Mice | Origin Image | Gray Image | Threshold Image |
|-------------|--------------|------------|-----------------|
| 1           | ![Image](image1) | ![Image](image2) | ![Image](image3) |
| 2           | ![Image](image4) | ![Image](image5) | ![Image](image6) |
| 3           | ![Image](image7) | ![Image](image8) | ![Image](image9) |
| 4           | ![Image](image10) | ![Image](image11) | ![Image](image12) |

Figure (2) The Statistical features for the Exposure for the control case.
| No. of Mice | Origin Image | Gray Image | Threshold Image |
|-------------|--------------|------------|-----------------|
| 5           | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| 6           | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
| 7           | ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) |
| 8           | ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) |

Figure (3) The Statistical features for the Exposure for the control case.