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## Immune Stimulation by Electroacupuncture at High Frequency ST36 Acupoint through SP on Hyperglycemia

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### Abstract

Electro acupuncture (EA) with a high frequency of 100 Hertz (HZ) at ST36 (Zusanli) acupoint is capable of stimulating interferon gamma (IFN- $\gamma$ ) as a phagocytosis activator through substance P (SP) in hyperglycemic conditions. EA at High frequency (100 Hz) ST36 acupoint administered in hyperglycemic rats daily for seven and fourteen days consecutively. Examination of SP and IFN- $\gamma$  levels using enzyme linked immunosorbent assay (ELISA) method with blood serum. The results indicated that EA with high frequency of 100 Hz on ST36 able to increase IFN- $\gamma$  through SP.

**Key words:** Electroacupuncture, ST36, substance P, interferon gamma

The ST36 acupoint is frequently used to stimulate immune system, evidenced by Longyun Chen and comrades study that EA at ST36 acupoint enhances the cytokine IFN- $\gamma$

(Chen *et al.*, 2017) but in hyperglycemic condition is still unknown where the phagocytosis disorder occurs (Jafar *et al.*, 2016). The SP neuropeptides released by motor neuron around the area of EA at ST36 acupoint thought to trigger the secretion of IFN- $\gamma$  through bonding with neurokinin receptors in phagocytic cells (Suvas, 2017; Mashaghi *et al.*, 2016; Vilisaar *et al.*, 2015).

### Materials and Methods

The study was conducted in veterinary laboratory with ethical clearance number: 2.KE.132.07.2019. 32 male Wistar rats aged 3 months (weight 150 grams per rat), acclimatized for 7 days in a cage with a temperature of 23°C and 12-hours light/dark cycle were fed standard pellet and water *ad libitum*. Hyperglycemic condition was made by injecting a single dose of STZ 60 mg per kilogram body weight intraperitoneally (blood sugar levels > 200 mg/dl) (King and Bowe, 2016; Nugraha *et al.*, 2019),

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**Table I.** SP and IFN- $\gamma$  levels in the control and treatment groups observed at the 7<sup>th</sup> and 14<sup>th</sup> day (mean  $\pm$  SD)

Variable	Control group				Treatment group			
	Observation at the 7 <sup>th</sup> day (n=8)	P	Observation at the 14 <sup>th</sup> day (n=8)	P	Observation at the 7 <sup>th</sup> day (n=8)	P	Observation at the 14 <sup>th</sup> day (n=8)	P
SP (pg/ml)	11.987 $\pm$ 2.317	.233*	11.916 $\pm$ 1.711	.589*	95.436 $\pm$ 11.382	.108*	171.565 $\pm$ 11.895	.765*
IFN- $\gamma$ (pg/mL)	8.721 $\pm$ .685	.801*	8.832 $\pm$ 1.007	.331*	24.116 $\pm$ 5.276	.097*	37.135 $\pm$ 2.741	.042*

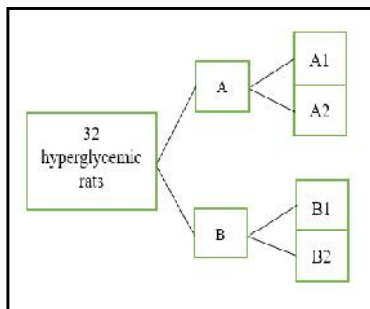
Notes: \*The data distribution was tested by using Shapiro-Wilk, normal distribution if  $P > .05$ ; SD = Std deviation; pg/ml = picograms per milliliter; n = number of subjects.

then divided randomly into control and treatment groups (Fig 1). The treatment group was inserted sterile acupuncture needle (length 3.0 cm, diameter 0.25 mm) at ST36 acupoint lateral to the anterior tubercle of the tibia, in left and right side at 5 mm depth, presented in Fig 2 (Wang *et al.*, 2016)its underlying mechanism on delayed-type hypersensitivity (DTH and connected with electroacupuncture stimulator (AES-05), dense sparse wave type, frequency 100 Hz, intensity 1 mA, and pulse width of 0.5 ms. The duration of EA was given each rat 30 minutes daily for 7 and 14 days consecutively. Blood serum was isolated from the rat’s heart blood then examined SP and IFN- $\gamma$  levels with ELISA method.

Results analysis include descriptive and inferential statistics using SPSS version 21.0 (IBM SPSS USA) with confidence interval (CI) 95%.

**Results and Discussion**

The treatment group until the fourteenth day had the highest average of SP and IFN- $\gamma$  levels compared to other groups (CI 95%), presented in



**Fig 1.** Study design. 32 hyperglycemic rats were divided into group A which observed for 7 days and group B which observed for 14 days. Subgroups A1 and B1 as controls, A2 and B2 which treated by EA at high frequency ST36 point.

the Table I.

ANOVA analysis shows a significant difference in the effects of EA at ST36 (the significance of  $P < .05$ ) for SP [ $F(3,28) = 674.774, P = .000$ ] and IFN- $\gamma$  [ $F(3,28) = 162.793, P = .000$ ]. Test of homogeneity of variance based on the mean obtained  $P = .000 (P < .05)$  at SP and IFN- $\gamma$  levels indicates a variance of the data is not homogeneous. Post hoc Games-Howell test ( $P < .05$ ) indicated a meaningful difference in SP levels between treatment groups up to the 14<sup>th</sup> day compared to the 7<sup>th</sup> day (mean difference = -76.130, std error = 5.821,  $P = .000$ ), as well as IFN- $\gamma$  levels between treatment group up to the 14<sup>th</sup> day compared to the 7<sup>th</sup> day (mean difference = -13.018, std error = 2.102,  $P = .000$ ).

Results of a correlation analysis of Pearson ( $r = .960, P = .000$  [significance at  $P < .01$  (2-tailed)] indicated the increased levels of IFN- $\gamma$  related significantly with the increase in SP levels in the treatment group with EA at ST36 with a frequency of 100 Hz.

The increase in IFN- $\gamma$  levels in our study is consistent with the findings of (Chen *et al.*, *loc. cit.*). EA with low frequency (between 2-15



**Fig 2.** The location of the EA at ST36 acupoint.



Hz) suppresses the release of SP which causes analgesia, but at high frequency (100 Hz) there is release of SP by neuron and the other cells (Han, 2003). Our study consistently shown that EA at high frequency (100 Hz) ST36 acupoint releases SP needed to modulate immune cell activity. Hence, this study corroborates the presence of an increase in blood levels of IFN- $\gamma$  accompanied by increased levels of SP in blood after the treatment of EA at high frequency ST36 acupoint (100 Hz) in hyperglycemia.

### Summary

Electroacupuncture at ST36 acupoint with a frequency of 100 Hz increases SP and IFN- $\gamma$  levels. An increase in IFN- $\gamma$  levels correlates statistically with an increase in SP levels.

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## Retrospective Study on Medicinal Disorders in Breeding Bulls

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### Abstract

Retrospective study was conducted to see the prevalence of different medical cases, their therapeutic management and recovery rate and management of bulls. The overall prevalence during three year period was found to be (28.71) and the percent of medical cases was 27.7. Breed wise classification indicated highest prevalence in HFX (9.39) followed by Indigenous (5.16). The values for HF, JY and JYX were nearly similar

(3.76, 3.29 and 3.76 respectively). The prevalence was least in buffalo bulls (2.35). It was observed that the recovery rate was recorded highest in buffalo followed by HF, HFX, JYX, JY & Indigenous bulls respectively.

**Key Words :** Retrospective study, Treatment, Breeding bulls

Non availability of sufficient number of breeding bull's for Artificial Insemination is hindering the quality and speed of genetic progress of the dairy livestock in the country.

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