ORIGINAL RESEARCH

Comparing The Effect Of Electroacupuncture And Glibenclamide On Blood Glucose Level And Histological Markers Of Pancreas In Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Background • Diabetes lowers the quality of life and leads to several complications. Glibenclamide is a commonly used step-two treatment in diabetes but it causes weight gain, hypoglycemia and cardiovascular problems. Electroacupuncture (EA) can enhance insulin sensitivity and reduce blood glucose levels.

Objectives • To compare the effects of EA plus glibenclamide (G) with single therapy by G or EA on blood glucose, pancreas volume, islet volume, ratio of islet volume to pancreas volume, apoptotic and beta cells numbers and body weight in diabetic rats.

Methods • Sixty adult male Wistar rats were randomly divided to 10 groups: 2 non-diabetic control groups and 8 diabetic groups (1 control and 7 experimental groups; D/G 2.5, D/G 5, D/G 10 mg/kg, EA, D/EA/G 2.5, D/EA/G 5, and D/EA/G 10). Diabetes was induced by intraperitoneal injection of 35 mg/kg streptozotocin with high-fat diet. At the end of the course, blood samples were obtained and pancreases were dissected.

Results • EA was as effective as D/G 5 and D/G 10 in all outcomes. Combination therapy of EA and glibenclamide 5 and 10 mg/kg resulted in a better glucose-lowering effect, greater islet volume and ratio of islet volume to pancreas volume than single therapies ($P < .05$). EA increased the pancreas volume as much as the combination therapies ($P > .05$).

Conclusion • Combination of EA and glibenclamide 5 showed the best effects on blood glucose, islet volume and ratio of islet to pancreas volume. Combination of EA and glibenclamide 2.5 illustrated the best effects on apoptotic and beta cell number of diabetic rats. (*Altern Ther Health Med.* 2020;26(S2):12-19.)

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INTRODUCTION

Diabetes is a lifestyle non-communicable endocrine or metabolic disorder of people considered as one of the most important global health issues that afflict both young and old in the world irrespective of their gender. The global prevalence of diabetes mellitus has increased in adults. Diabetes mellitus not only decreases quality of life and life anticipation, but is also a serious reason of several microvascular and macrovascular complications that lead to blindness, renal failure, myocardial infarction and stroke. Diabetes characterized by impaired glucose utilization that leads to chronic hyperglycemia which is a result of the body's inability to produce or make use of insulin.[1] Long-term hyperglycemia can cause damage to multiple systems, and these complications cause mortality.[2] The prevalence of diabetes in Middle East of Asia is 7.7%.[3] In type I diabetes pancreatic cells, which are responsible for making insulin, affected by inhibiting the immune response. However, type II diabetes, characterized by hyperglycemia resulting from insulin resistant and β-cell dysfunction.[4] Type II diabetes affected 382 million people globally in 2013, and it is estimated to rise up to 592 million by 2035.[5] The current therapy of type 2 diabetes mellitus

(T2DM) is a help to control hyperglycemia within the normal level (between 4.4 to 7.2 mmol/L) and stop the progression of its related complications but failure of pharmacological drugs is not uncommon.[6]

One of the best current methods for creating diabetes in animals is injecting drugs like Streptozotocin (STZ). STZ, a monofunctional nitrosourea derivative, inflate and ultimately degenerate the Langerhans islets beta cells.[7]

One of the chemical drugs that are currently used for diabetes is glibenclamide as a step-two treatment. Glibenclamide is a second-generation sulfonylurea that reduces blood glucose by increasing insulin secretion from beta cells of the pancreas.[8] It is given to diabetic patients as a 5-milligram tablet.[9] It has a long duration of action but confer an increased risk of prolonged hypoglycemia, cardiovascular effects and weight gain.[10,11]

As a traditional Chinese medicine treatment, acupuncture has been practiced in China from the past to now. It is also a very significant therapeutic method in the complementary medicine. Electroacupuncture (EA) is a modification of this method. It stimulates acupoints with electrical current and seems to have more consistently reproducible results than manual manipulations.[12] EA is also easy to apply, the cost is low and its side effects are minimum.[13] Some observations demonstrate that EA may help diabetic patients by accelerating gastric emptying and reducing symptom severity.[14]

ST36 (Zusanli), CV4 (Guanyuan) and CV12 (Zhongwan) are several acupoints that have been used for treatment of diabetes.[15] Chang Suggested that stimulation of CV12 results in meaningfully greater plasma glucose-lowering effects than stimulation of adjacent non-acupuncture points.[16] The glucose-lowering effects of EA applied to ST36 involves the release of serotonin and β-endorphins that raise insulin production and stimulate the cholinergic nerves and inducing the up-regulation of the insulin signaling proteins IRS-1 and AKT-2.[17,18] Application of EA to the ST36 or CV12 acupoints improves insulin sensitivity in rats although the mechanisms of action are not investigated.[19,20]

The aim of the present study is to investigate whether the combination of EA and glibenclamide would have effectiveness in blood glucose, body weight, pancreas volume, islet volume, apoptotic and beta cell number in an animal model and determining the most effective dose of glibenclamide in combination with EA.

METHODS

Animals

Male Wistar rats weighting 250 to 300 g were taken from the animal lab of Mashhad University of medical science, Iran. They were housed in an air-conditioned colony room at 22 ± 2° C with 12 hours light: 12 hours dark cycles and they were fed ad libitum with normal laboratory chow. Before testing for blood glucose levels, rats were fasted overnight (at least 12 hours) with free access to water. Procedures involving animals and their care were conducted in conformity with National Institute of Health (NIH) guidelines for the care and use of laboratory animals. All attempts were made to reduce animal suffering and minimize the number of animals used.

Induction of experimental diabetes

At the beginning of the new millennium, Reed reported a new model of inducing diabetes in rats.[21] This model is today known as the HFD/STZ rat, as well as by other names (e.g. high energy/ STZ rat). The rats were fed on a high-fat diet (HFD). After 4 weeks of dietary manipulation, DM rats were administrated with an intraperitoneal injection of STZ (35 mg/kg, dissolved in pH 4.5 citrate buffer). At 72 h after STZ injection in fasting state, blood samples were collected from the tail vein and blood glucose levels were measured by a portable glucometer (Easy Gluco TM, Infopia, Korea). Blood glucose level greater than 16.7 mmol/L was taken as the standard for successful establishment of DM model.[22,23]

Glibenclamide

Glibenclamide was dissolved in normal saline 0.9% (w/v) and this study included doses of glibenclamide used clinically, ie, 2.5, 5 and 10 mg/kg body weight.

EA

ST36, CV4 and CV12 acupoints were punctured in this study. Location of these points in rat was found earlier by Romita (24). The ST36 acupoint is located on the anterior tibia muscle nearly 5mm below the knee. EA was also applied at the CV12 and CV4 acupoints. CV12 is 9/14 above the pubic crest of the distance between the top of the xiphoid process and the pubic crest, whereas the CV4 acupoint is 2/14 of this distance above the pubic crest.[16] Acupoints were punctured in a vertical manner with disposable needles of 0.25 mm diameter and 40 mm length (Suzhou Acupuncture & Moxibustion Appliance Co, China). After a 5-min needling time, The points were electrically motivated with a low frequency of 2 Hz twice a week for 3 weeks as previously used by Steiner-Victorin and Manni.[25,26] The intensity (1.0 to 1.5 mA) was monitored by checking for local muscle contractions to reflect the activation of muscle–nerve afferents. During each electroacupuncture treatment, rats were sedated with a mixture of ketamine (60 mg/kg) and xylazine (6 mg/kg).

Experimental design

The animals were randomly divided into ten groups (n = 6) and the treatment of them began 72 hours after the induction of diabetes. The animals were treated for 3 weeks as follows: (1) Sham control group (cont. salin): Rats of this group received once daily gavages vehicle with 0.9% NaCl. (2) Ketamine and xylazine control group (cont. keta): Rats of this group received ketamine and xylazine by i.p. injection two times in a week. (3) The Diabetic control group (Diabetic): in this group and the groups will mention below, diabetes induced by 3 weeks high-fat diet and then i.p. injection of STZ. (4) Glibenclamide treated group (D/G 2.5): Rats of this

group received Glibenclamide once daily (2.5 mg/kg body weight) after the induction of diabetes. (5) Glibenclamide treated group (D/G 5): Rats of this group received Glibenclamide once daily (5 mg/kg body weight) after the induction of diabetes. (6) Glibenclamide treated group (D/G 10): Rats of this group received Glibenclamide once daily (10 mg/kg body weight) after the induction of diabetes.(7) EA treated group(D/EA): Rats of this group received EA two times in a week after the induction of diabetes. (8) EA With Glibenclamide (2.5 mg/kg body weight)(D/EA/G 2.5): Rats of this group received EA two times in a week and Glibenclamide once daily (2.5 mg/kg body weight) after the induction of diabetes. (9) EA With Glibenclamide (5 mg/kg body weight)(D/EA/G 5): Rats of this group received EA two times in a week and Glibenclamide once daily (5 mg/kg body weight) after the induction of diabetes.(X) EA With Glibenclamide (10 mg/kg body weight)(D/EA/G 10): Rats of this group received EA two times in a week and Glibenclamide once daily (10 mg/kg body weight) after the induction of diabetes.

During each EA treatment, rats were sedated by a mixture of ketamine (60 mg/kg) and xylazine (6 mg/kg).The points that we selected for EA were ST36, CV12 and CV4 and needles were injected vertically and then electrical current connected to the needles for 30 minutes with low frequency of 2 Hz and intensity of 1.5 mA.

The results of this study will participate to a better understanding that whether EA has additional effects to glibenclamide for controlling the hyperglycemic condition and also if we want to suggest EA with glibenclamide, which dose of this drug is more effective?

Histology

After blood samples collection on day 21, pancreases were immediately removed, rinsed in ice-cold saline, and fixed in 10% neutral buffered formalin (vol/vol), dehydrated and embedded in paraffin. Following embedding, 5-µm-thick sections were cut, stained with Hematoxylin and Eosin, Modified Gomori Aldehyde Fuchsin and for TUNEL technique.

TUNEL Assay

At the first, sections were deparaffinised with the xylene, rehydrated through descending concentrations of ethanol and then rinsed in PBS (0.1M) for 15 min, the sections treated with 3% H2O2 in methanol in the darkness at room temperature for 10 min to inactivate endogenous peroxidase and then rinsed in PBS (0.1M) for 15 min. In the next stage, the sections were treated with proteins K (Roche, Germany) at room temperature for 20 min. After rinsing in PBS (0.1M) for 15 min, the sections were incubated in the labeling reaction mixture (TUNEL Kit (Roche, Germany)) at 4° C overnight. The sections were rinsed in PBS (0.1M) for 15 min and then incubated in Convertor-POD at room temperature for 1 hour. In next step, the sections were rinsed in PBS (0.1M) for 15 min. Finally; the sections were treated with 0.03% Diaminobenzidine (DAB) (Sigma, USA) solution for 15 min and then washed with running water. Counterstaining was done by Hematoxylin. After dehydration and clearing, the sections were mounted with cover slip.[27] Apoptotic nuclei were dark brown in this method.

Statistical Analysis

Data are expressed as mean ± SD. SPSS 17 software was used for statistical analysis. Statistical analysis was performed using one-way ANOVA followed by Tukey post-hoc test for multiple comparisons. The *P* values less than .05 were considered statistically significant.

RESULTS

Body Weight At The Start Of Intervention

In 72 hours after diabetes induction there was no significant difference ($P > .05$) in body weight between different groups (Figure1a).

Body Weight At The End Of Intervention

At the end of intervention, the body weight of the diabetic group significantly reduced in comparison to control groups ($P < .001$). Treatment with glibenclamide improved the body weight of the diabetic rats. D/G 2.5 significantly increased the body weight as compared to diabetic group ($P = .008$). Both D/G 5 and D/G 10 meaningfully enhanced the body weight in comparison to diabetic group ($P < .001$). There was no significant difference between D/EA and also D/EA/G in comparison to diabetic group ($P > .05$). There was significant difference between D/G 2.5 and D/EA ($P = .008$). D/G 5 and D/G 10 showed significant differences as compared to D/EA ($P < .001$). Combination treatment groups had not significant differences in comparison to D/EA and D/G 5 ($P > .05$; Figure1b).

Blood Glucose Level At The Start Of Intervention

At the start of our intervention diabetes caused a significant increase in blood glucose as compared with control groups ($P < .001$). There were not meaningful differences in experimental groups in comparison to diabetic group ($P > .05$). Glibenclamide and combination treatment groups had not significant differences with D/EA ($P > .05$). There were not meaningful differences between combination therapies and D/G 5 ($P > .05$; Figure1c).

Blood Glucose Level At The End Of Intervention

At the end of the interventions, blood glucose was measured in all of the groups. Diabetic group showed a significant difference in comparison to control groups ($P < .001$). Except D/G 2.5, other experimental groups had significant differences as compared with diabetic group ($P < .001$). Between glibenclamide groups, just D/G 2.5 had significant difference as compared to D/EA ($P < .001$). D/EA/G 5 and D/EA/G 10 showed significant differences as compared with D/EA ($P = .003$ and $P = .006$ respectively). Among the combination therapies there was significant difference between D/EA/G 5 and D/EA/G 10 in comparison to D/G 5 ($P < .001$; Figure 1d).

Figure 1a. Comparisons of body weight in different groups at the start of interventions (Mean ± SD).

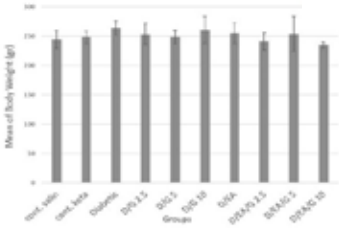
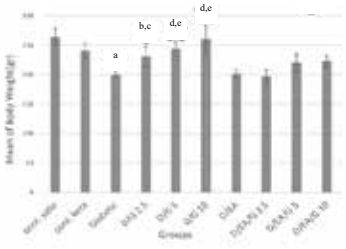
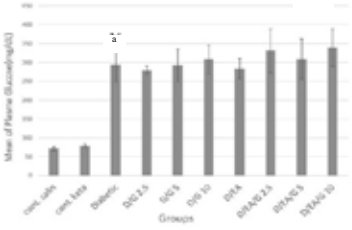


Figure 1b. Comparisons of body weight in different groups at the end of interventions (Mean ± SD).



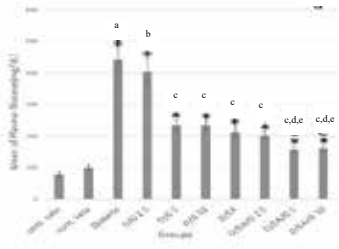
[a]$P < .001$ compared to sham control groups
[b]$P < .05$ compared to alone acupuncture group
[c]$P < .05$ compared to diabetic control group
[d]$P < .001$ compared to diabetic control group
[e]$P < .001$ compared to alone acupuncture group

Figure 1c. Comparisons of blood glucose level in different groups at the start of interventions (Mean ± SD).



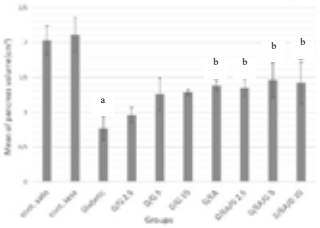
[a]$P < .001$ compared to sham control groups

Figure 1d. Comparisons of blood glucose level in different groups at the end of interventions (Mean ± SD).



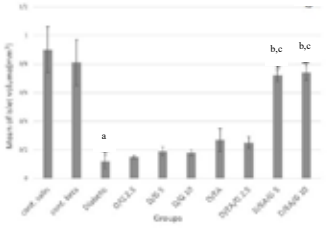
[a]$P < .001$ compared to sham control groups
[b]$P < .001$ compared to D/EA group
[c]$P < .001$ compared to diabetic control group
[d]$P < .05$ compared to D/EA group
[e]$P < .001$ compared to D/G 5 group

Figure 2a. Comparisons of pancreas volumes in different groups at the end of interventions (Mean ± SD).



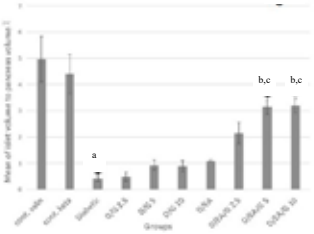
[a]$P < .001$ compared to sham control groups,
[b]$P < .05$ compared to diabetic control group.

Figure 2b. Comparisons of pancreatic islet volumes in different groups at the end of interventions (Mean ± SD).



[a]$P < .001$ compared to sham control groups
[b]$P < .001$ compared to diabetic control group
[c]$P < .05$ compared to D/G 5 group.

Figure 2c. Comparisons of the ratio of islet volumes to pancreas volumes in different groups at the end of interventions (Mean ± SD).

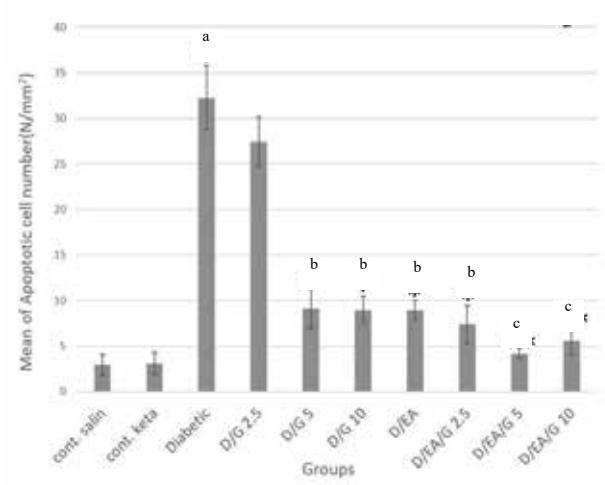


[a]$P < .001$ compared to sham control groups
[b]$P < .001$ compared to diabetic control group
[c]$P < .05$ compared to D/G 5 group.

Pancreas Volume

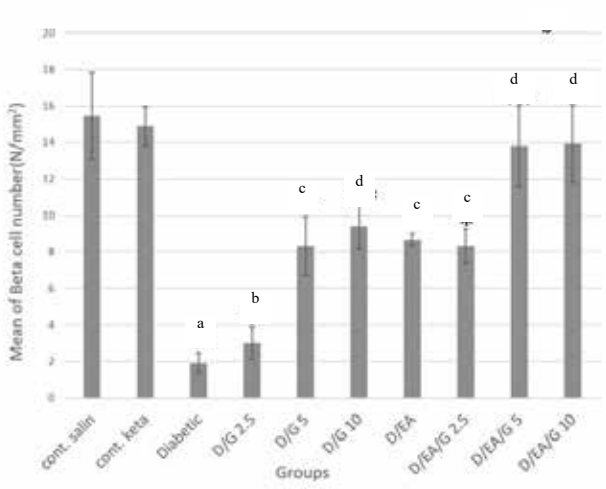
Diabetes caused a significant decrease in pancreas volume as compared with control groups ($P < .001$). All of the treatment groups increased the pancreas volumes but just 4 treatment groups as mentioned below had significant differences. D/EA and D/EA/G 2.5 significantly enhanced the pancreas volume in comparison to diabetic group ($P = .02$). Also, there was a significant difference between D/EA/G 5 and D/EA/G 10 as compared to diabetic group ($P = .003$ and $P = .007$ respectively). All glibenclamide groups and combination treatment groups had no significant

Figure 3a. Comparisons the mean of apoptotic cells number in different groups at the end of interventions (Mean ± SD).



[a]$P < .001$ compared to sham control groups
[b]$P < .05$ compared to diabetic control group
[c]$P < .001$ compared to diabetic control group

Figure 3b. Comparisons the mean of beta cells number in different groups at the end of interventions (Mean ± SD).



[a]$P < .001$ compared to sham control groups
[b]$P < .05$ compared to D/EA
[c]$P < .05$ compared to diabetic control group
[d]$P < .001$ compared to diabetic control group

differences in comparison to D/EA ($P > .05$). Among the combination therapies there was no significant difference in comparison to D/G 5 group ($P > .05$; Figure 2a).

Pancreatic Islet Volume

Based on inducing diabetes by STZ a significant decrease occurred in pancreatic islet volumes in comparison to control groups ($P < .001$). All of the treatments increased the volumes of islets as compared to diabetic group but just D/EA/G 5 and D/EA/G 10 significantly increased the islet volumes ($P < .001$). Glibenclamide groups had no significant differences in comparison to D/EA ($P > .05$). Using combination treatment was effective for enhancing the volumes of islets but these groups had no meaningful differences with D/EA ($P > .05$). Among the combination therapies there was significant difference between D/EA/G 5 and D/EA/G 10 in comparison to D/G 5 ($P = .009$; Figure 2b).

The Ratio Of Islet Volumes To Pancreas Volumes

Same as islet volume and pancreas volume, inducing diabetes made a significant decrease in the ratio of islet volumes to pancreas volumes in comparison to control groups too ($P < .001$). All of the experimental groups increased this ratio. Between treatment groups, D/EA/G 5 and D/EA/G 10 significantly enhanced this ratio in comparison to diabetic group ($P = .009$). Glibenclamide groups had no significant differences as compared to D/EA ($P > .05$). Using combination of glibenclamide and EA was effective for enhancing this ratio but these groups didn't show significant differences in comparison to D/EA ($P > .05$). Among the combination therapies there was significant difference between D/EA/G 5 and D/EA/G 10 in comparison to D/G 5 ($P = .04$; Figure 2c).

Mean of Apoptotic Cell Number

Diabetes caused a meaningful enhance in the number of apoptotic cells (per unit area) as compared to control groups ($P < .001$). Except D/G 2.5, all of the treatment groups showed a significant decrease. D/G 5, D/G 10, D/EA and D/EA/G 2.5 demonstrated significant decrease as compared to diabetic group ($P = .009$, $P = .007$, $P = .007$ and 0.003 respectively). D/EA/G 5 and D/EA/G 10 had significant decrease the number of apoptotic cells in comparison to diabetic group ($P < .001$). Glibenclamide groups had no significant difference with D/EA ($P > .05$). There was no meaningful difference between combination experimental groups and D/EA or D/G 5 ($P > .05$; Figure 3a, 4).

Mean of Beta cell number

Following diabetes induced by STZ, the number of beta cells significantly reduced as compared to control groups ($P > .001$). Among the treatment groups, only D/G 2.5 didn't show significant increase in beta cells number in comparison to diabetic groups ($P > .05$). D/G 5, D/EA and D/EA/G 2.5 showed significant increase as compared to diabetic group ($P = .004$, $P = .002$ and $P = .004$ respectively). D/G 10, D/EA/G 5 and D/EA/G 10 demonstrated a significant increase in comparison to diabetic group ($P < .001$). Among all of experimental groups just D/G 2.5 had significant difference with D/EA ($P = .009$). There was no meaningful difference between combination experimental groups and D/G 5 ($P > .05$; Figure 3b, 5).

Figure 4. Photomicrographs showing the apoptotic cells in the pancreatic islet in different groups.

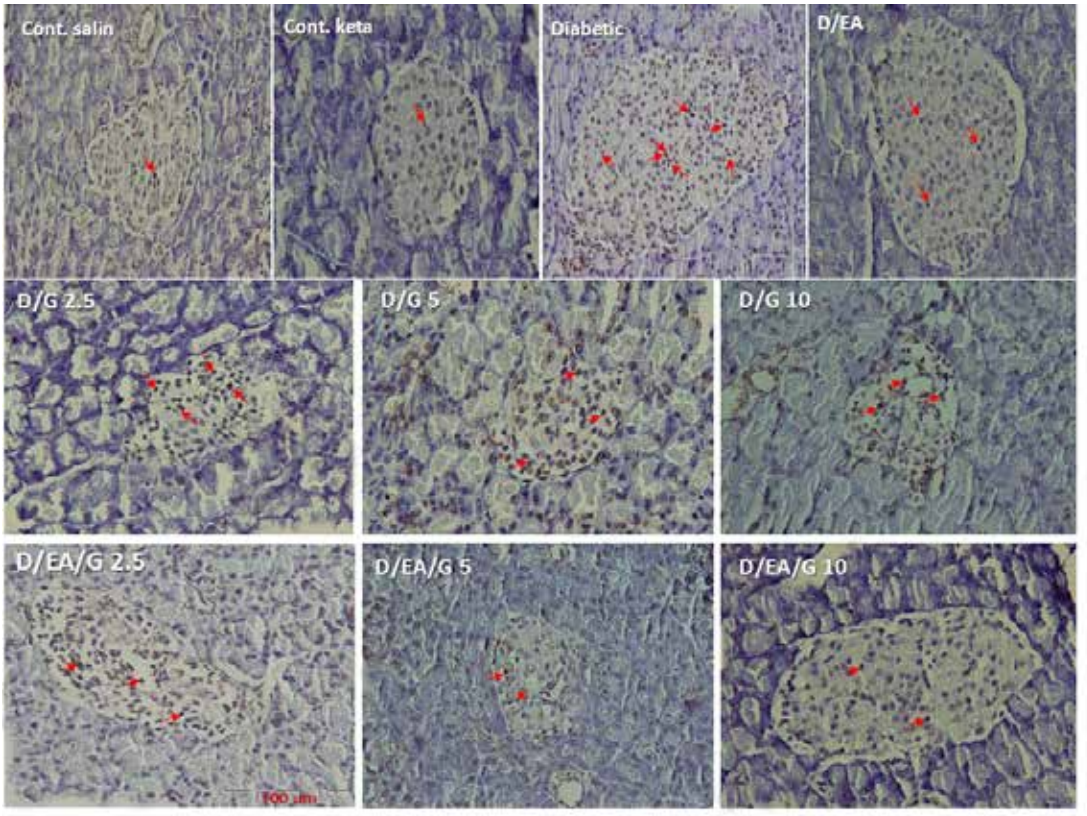
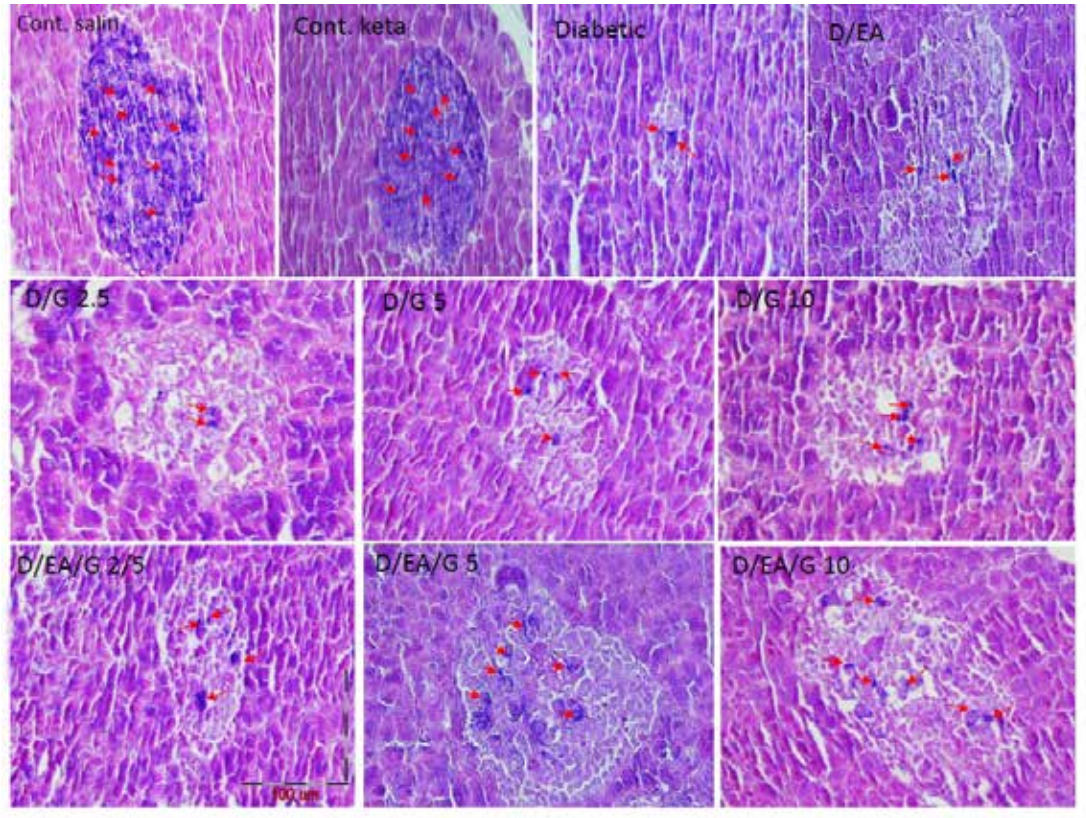


Figure 5. Photomicrographs showing the beta cells in the pancreatic islet in different groups.



DISCUSSION

We compared the effect of EA, glibenclamide and combination of them on blood glucose level, pancreas volume, islet volume, ratio of islet volume to pancreas volume, number of beta cells, number of apoptotic cells and body weight in diabetic rats.

Our results suggested that a combination of EA and glibenclamide either 5 or 10 mg/kg showed a better effect than EA alone and glibenclamide alone on blood glucose, the pancreas islet volume and the ratio of islet volume to pancreas volume. EA and combination therapies significantly increased the pancreas volume compared to no treatment diabetic group, with no significant difference between EA and combination therapies. All of the experimental groups reduced the number of apoptotic cells and increased the number of beta cells significantly compared to the non-treatment diabetic group except D/G 2.5. There was no significant difference between single therapy groups and combination therapy groups in reducing the number of apoptotic cells and increasing the number of beta cells except for D/G 2.5 for beta cell number when comparing to EA alone. The effect of D/G 2.5 was significantly less than EA alone in increasing the number of beta cells. Single therapy by glibenclamide significantly increased the body weight of diabetic rats while combination therapies and EA did not.

In blood glucose, islet volume and ratio of islet volume to pancreas volume, combination therapy of EA and glibenclamide 5 mg/kg and 10 mg/kg led to better results than single therapies and effect were near to normal range and because of choosing fewer doses, combination of EA and glibenclamide 5 mg/kg looks to be a better choice for them. In pancreas volume, EA alone was as effective as combination therapy. In apoptotic and beta cell number, there were no significant differences between combination therapies and as the fewer doses are preferable, combination of EA and glibenclamide 2.5 mg/kg seems to be a better choice for them.

Our study approved the results of previous studies about the effects of EA at ST36 on blood glucose level.[18,19,28] We obtained similar results about the effects of EA at CV4 and CV12 on blood glucose level with previous studies.[20,29-31] The type of diabetes and results of EA on lowering blood glucose level and insufficiency of EA on body weight were similar in our study and in nakamora's study.[31] The effects of EA at CV4 and ST36 on blood glucose level were similar in our study and study by Liang and like our study, Liang used low frequency EA.[32]

There was no study concentrating on histology except the study of Jiang in 2011 that measured the blood glucose level and the volume of pancreas islets and obtained similar results as ours. They removed pancreas, stained with Hematoxylin and Eosin and observed no significant difference with EA on the volume of islets.[33]

We found only one study examining the combination therapy. Liao in 2015 showed that combination therapy of EA and metformin led to better effects on lowering the blood glucose in comparison to single therapy in diabetic rats.[34] Our study approved the subject that combination treatment has better effect as compared to single therapy.

ATP-sensitive potassium channels (KATP) are extremely divided in muscle, beta cells of pancreas and the brain. The mechanism of glibenclamide in the treatment of diabetes mellitus is as a result of its inhibition of KATP in pancreatic beta cells, which leads to depolarization of beta cell plasma membrane and activation of Voltage-gated calcium channels. Calcium influx releasing insulin from beta cells.[35,36] Glibenclamide also has insulin-like effects on the metabolism of glucose. On the other hand, the drug decreases glucogenesis and glycogenolysis in the body cells and then it reduces the level of blood glucose.[37] Some mechanisms have been suggested for the effect of EA in diabetes and they have no similarity with the mechanisms of glibenclamide. Insulin resistance is associated with hyperactivity in the sympathetic nervous system, which causes a pre-inflammatory condition and, as a result, leads to type 2 diabetes.[38] The activation of the cholinergic nerves by EA improves the hypoglycemic effect of insulin.[39] EA could increase the SIRT1 protein expression and up-regulate PGC-1α, NRF1, and ACOX gene expression which in turn, may enhance mitochondrial biogenesis and fatty acid oxidation and up-regulate insulin-associated signal transduction with subsequent improvement in insulin resistance.[32]

To the best of our knowledge, this study is the first study that investigated the effect of combined glibenclamide and EA. It is also the only study that concentrated on the pancreas histological changes due to EA by specific staining of beta cells by Gomori Aldehyde Fuchsin and apoptotic cells by TUNNEL assay. There was only one other histological study on EA done by Jiang et al, which used H&E just for pancreas islet volume while we used better staining method for 5 histological parameters.[33] Some of the limitations of our study are the lack of examination of gene and protein expression and the lack of measurement of insulin levels. Future studies that combine the investigation of gene and protein expression with histological changes created by combination therapy of glibenclamide and EA are suggested.

CONCLUSION

This study suggested that use of EA has same effects with glibenclamide 5, 10 mg/kg but simultaneous application of EA and glibenclamide 5, 10 mg/kg shows the best effects in on blood glucose, islet volume and ratio of islet volume to pancreas volume. Using combination therapies positively enhanced apoptotic and beta cell number in comparison to single therapy but this improvement was not meaningful.

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CONFLICT OF INTEREST

None

REFERENCES

1. Wilcox G. Insulin and insulin resistance. *Clinical biochemist reviews*. 2005;26(2):19.
2. Calcutt NA, Cooper ME, Kern TS, Schmidt AM. Therapies for hyperglycaemia-induced diabetic complications: from animal models to clinical trials. *Nature Reviews Drug Discovery*. 2009;8(5):417-30.
3. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice*. 2011;94(3):311-21.
4. Hull RL, Westermark GT, Westermark P, Kahn SE. Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. *The Journal of clinical endocrinology & metabolism*. 2004;89(8):3629-43.
5. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice*. 2014;103(2):137-49.
6. Irons BK, Minze MG. Drug treatment of type 2 diabetes mellitus in patients for whom metformin is contraindicated. *Diabetes, metabolic syndrome and obesity: targets and therapy*. 2014;7:15.
7. Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia*. 2008;51(2):216-26.
8. Groop L, Groop P-H, Stenman S, Saloranta C, Tötterman K-J, Fyhrquist F, et al. Comparison of pharmacokinetics, metabolic effects and mechanisms of action of glyburide and glipizide during long-term treatment. *Diabetes Care*. 1987;10(6):671-8.
9. Capasso F, De Fusco R, Fasulo M, Lembo M, Mascolo N, Menghini A. Antipyretic and antibacterial actions of Teucrium polium (L.). *Pharmacol Res Commun*. 1984;16(1):21-9.
10. Jönsson A, Karlsson M, Melander A. Concentration-effect relations of glibenclamide and its active metabolites in man: modelling of Pharmacokinetics and Pharmacodynamics. *British journal of clinical pharmacology*. 1997;43(4):373-81.
11. Meier J, Gallwitz B, Schmidt W, Mügge A, Nauck M. Is impairment of ischaemic preconditioning by sulfonylurea drugs clinically important? *Heart*. 2004;90(1):9-12.
12. Chi-Sen C, Chung-Wang K, Chun-Ying W, Chen G-H. Effect of electrical stimulation on acupuncture points in diabetic patients with gastric dysrhythmia: a pilot study. *Digestion*. 2001;64(3):184.
13. Cui K, Li W, Gao X, Chung K, Chung J, Wu G. Electro-acupuncture relieves chronic visceral hyperalgesia in rats. *Neuroscience letters*. 2005;376(1):20-3.
14. Ouyang H, Yin J, Wang Z, Pasricha PJ, Chen J. Electroacupuncture accelerates gastric emptying in association with changes in vagal activity. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 2002;282(2):G390-G6.
15. Yang J, Yang Y, Chen J-M, Liu W-Y, Wang C-H, Lin B-C. Effect of oxytocin on acupuncture analgesia in the rat. *Neuropeptides*. 2007;41(5):285-92.
16. Chang S, Lin J, Chi T, Liu I, Cheng J. An insulin-dependent hypoglycaemia induced by electroacupuncture at the Zhongwan (CV12) acupoint in diabetic rats. Diabetologia. 1999;42(2):250-5.
17. Chang S-L, Tsai C-C, Lin J-G, Hsieh C-L, Lin R-T, Cheng J-T. Involvement of serotonin in the hypoglycemic response to 2Hz electroacupuncture of zusanli acupoint (ST36) in rats. *Neuroscience letters*. 2005;379(1):69-73.
18. Lee Y-C, Li T-M, Tzeng C-Y, Chen Y-I, Ho W-J, Lin J-G, et al. Electroacupuncture at the Zusanli (ST-36) acupoint induces a hypoglycemic effect by stimulating the cholinergic nerve in a rat model of streptozotocine-induced insulin-dependent diabetes mellitus. *Evidence-Based Complementary and Alternative Medicine*. 2011;2011.
19. Chang S-L, Lin K-J, Lin R-T, Hung P-H, Lin J-G, Cheng J-T. Enhanced insulin sensitivity using electroacupuncture on bilateral Zusanli acupoints (ST 36) in rats. *Life sciences*. 2006;79(10):967-71.
20. Ishizaki N, Okushi N, Yano T, Yamamura Y. Improvement in glucose tolerance as a result of enhanced insulin sensitivity during electroacupuncture in spontaneously diabetic Goto-Kakizaki rats. *Metabolism*. 2009;58(10):1372-8.
21. Reed M, Meszaros K, Entes L, Claypool M, Pinkett J, Gadbois T, et al. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism*. 2000;49(11):1390-4.
22. Skovsø S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *Journal of diabetes investigation*. 2014;5(4):349-58.
23. Winzell MS, Ahrén B. The High-Fat Diet–Fed Mouse. *Diabetes*. 2004;53(suppl 3):S215-S9.
24. Romita VV, Yashpal K, Hui-Chan CW, Henry JL. Intense peripheral electrical stimulation evokes brief and persistent inhibition of the nociceptive tail withdrawal reflex in the rat. *Brain research*. 1997;761(2):192-202.
25. Stener-Victorin E, Aloe L, Manni L, Janson PO, Cajander S, Lundeberg T, et al. Steroid-induced polycystic ovaries in rats: effect of electro-acupuncture on concentrations of endothelin-1 and nerve growth factor (NGF), and expression of NGF mRNA in the ovaries, the adrenal glands, and the central nervous system. *Reproductive Biology and Endocrinology*. 2003;1(1):33.
26. Manni L, Holmäng A, Lundeberg T, Aloe L, Stener-Victorin E. Ovarian expression of alpha (1)-and beta (2)-adrenoceptors and p75 neurotrophin receptors in rats with steroid-induced polycystic ovaries. *Autonomic Neuroscience*. 2005;118(1):79-87.
27. KhoshdelSarkarizi H SGaRA. Morphometrical study of tunel-positive cells in the pancreatic islets of diabetic rats treated with teucriumpolium l. extract and glibenclamide. *International Journal of Current Life Sciences*. 2014;4(9):7149-55.
28. Pai H-C, Tzeng C-Y, Lee Y-C, Chang C-H, Lin J-G, Cheng J-T, et al. Increase in plasma glucose lowering action of rosiglitazone by electroacupuncture at bilateral Zusanli acupoints (ST. 36) in rats. *Journal of Acupuncture and Meridian Studies*. 2009;2(2):147-51.
29. Tzeng C-Y, Lee Y-C, Ho T-Y, Chen Y-I, Hsu T-H, Lin J-G, et al. Intracellular signalling pathways associated with the glucose-lowering effect of ST36 electroacupuncture in streptozotocin-induced diabetic rats. *Acupuncture in Medicine*. 2015:acupmed-2014-010718.
30. Peplow PV, McLean GT. Repeated electroacupuncture: an effective treatment for hyperglycemia in a rat model. *Journal of acupuncture and meridian studies*. 2015;8(2):71-6.
31. Nakamura H, Ishigami T, Kawase Y, Yamada A, Minagawa M, Fukuta H, et al. Effects of acupuncture stimulation on blood glucose concentration in the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, an animal model for type-2 diabetes mellitus. *Medical science monitor basic research*. 2014;20:70.
32. Liang F, Chen R, Nakagawa A, Nishizawa M, Tsuda S, Wang H, et al. Low-frequency electroacupuncture improves insulin sensitivity in obese diabetic mice through activation of SIRT1/PGC-1 in skeletal muscle. *Evidence-Based Complementary and Alternative Medicine*. 2011;2011.
33. Jiang Y, Ning Y, Liu Y, Wang Y, Zhang Z, Yin L, et al. Effects of preventive acupuncture on streptozotocin-induced hyperglycemia in rats. *Journal of endocrinological investigation*. 2011;34(10):e355-e61.
34. Liao H-Y, Sun M-F, Lin J-G, Chang S-L, Lee Y-C. Electroacupuncture plus metformin lowers glucose levels and facilitates insulin sensitivity by activating MAPK in steroid-induced insulin-resistant rats. *Acupuncture in Medicine*. 2015:acupmed-2014-010724.
35. Ashcroft FM. K(ATP) channels and insulin secretion: a key role in health and disease. Biochemical Society transactions. 2006;34(Pt 2):243-6.
36. Nichols CG. KATP channels as molecular sensors of cellular metabolism. *Nature*. 2006;440(7083):470-6.
37. andZahra Shirdel RM. Antihyperglycemic and Antihyperlipidemic effects of Cornus mas extract in diabetic rats compared with glibenclamide. 2012.
38. Greenfield JR, Campbell LV. Role of the autonomic nervous system and neuropeptides in the development of obesity in humans: targets for therapy? *Current pharmaceutical design*. 2008;14(18):1815-20.
39. Lee Y-C, Li T-M, Tzeng C-Y, Cheng Y-W, Chen Y-I, Ho W-J, et al. Electroacupuncture-induced cholinergic nerve activation enhances the hypoglycemic effect of exogenous insulin in a rat model of streptozotocin-induced diabetes. *Experimental diabetes research*. 2011;2011.