Clinical Studies

A Randomized Cross-over Clinical Study Evaluating the Effect of Acupuncture on Blood Pressure, Blood Glucose and Hematological Parameters in Healthy Dogs

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ABSTRACT

Acupuncture is being used as a medical treatment for diseases such as diabetes mellitus, hypertension and immune disorders due to documented positive effects on blood pressure, blood glucose and hematological parameters in research studies and human clinical trials. This study was designed as a randomized crossover experiment to evaluate the effects of acupuncture on blood pressure, blood glucose and hematological parameters (hematocrit, hemoglobin, white blood cell count) in healthy canines. The study recruited 100 healthy dogs which were divided into 2 groups of 50 dogs (Group A, Group B). Dry needle stimulation was performed at acupuncture points LI-4, ST-36, GB-34 and SP-6 by bilateral insertion of needles which remained in place for 20 minutes. Blood pressure, blood glucose and hematology measurements were performed as a repeated measures design (baseline, 20 minutes, 60 minutes, 7 days) where study animals crossed over from 1 treatment (real acupuncture points) to the other treatment (sham acupuncture points) during the trial. Needle insertion performed at real acupoint locations had a significant effect (p<0.0001) on lowering blood glucose values, increasing the WBC count (white blood cells) and lowered blood pressure measurement whereas needle insertion at sham sites had no statistically significant effects on these parameters. Hematocrit and hemoglobin measurements did not have statistically significant changes associated with acupuncture stimulation. The results of this study suggest that acupuncture is a beneficial cost-effective therapeutic modality for disorders affecting the immune system, diabetes mellitus and hypertension.

ABBREVIATIONS

CBC	Complete Blood Count
WBC	White Blood Cell
HCT	Hematocrit
HGB	Hemoglobin
NSAIDS	Nonsteroidal anti-inflammatory drugs
CNS	Central nervous system

Over the past 5 decades there has been an increasing volume of evidence of beneficial effects attained from the use of acupuncture in disease syndromes such as hypertension, diabetes mellitus and immune disorders primarily in humans.[1-17]   Vascular hypertension is a particularly common disease occurring in humans and eventually leads to pathological changes in the brain, heart and kidney. In the past 15 years, there has been increased recognition of hypertension in dogs and cats.[18] It is associated with a number of diseases in animals such as diabetes mellitus, Cushing's disease, obesity, hypothyroidism and some drugs (NSAIDs, steroids).[18] Untreated, chronically sustained increases in blood pressure, similar to humans, results in injury to the kidneys, eyes, heart and central nervous system (CNS). Treating hypertension in animals, as well as humans, is difficult and usually lifelong once diagnosed. Acupuncture has been reported to reduce blood pressure and has been documented as an effective primary or adjunct treatment with drugs in hypertensive human patients.[16, 19-22]

Other important disease syndromes in humans, such as diabetes mellitus, have demonstrated beneficial effects associated with acupuncture through the reduction of blood glucose levels.[12-14, 17, 23-27] A number of current studies, involving mechanistic, preclinical and clinical trials substantiate the observations made in earlier studies of the antihyperglycemic effect of acupuncture treatment.[12-14, 28, 29]   In addition to metabolic disease syndromes, acupuncture has demonstrated bone marrow and immunomodulatory effects. Current studies have been predominately associated with effects in humans or mechanistic preclinical studies with effects on dogs largely ignored in the literature.[30-32, 15]

The aim of this study was to document the effect of acupuncture stimulation in dogs on disease conditions that have responded well to acupuncture in humans. Blood pressure, blood glucose, white blood cell count, hematocrit and hemoglobin measurements were collected

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from normal healthy adult dogs following acupuncture treatment in a cross over study which then treated the same dog with sham acupuncture in a second arm of the clinical trial. The hypothesis for this study was that dogs would respond in a similar fashion as humans and would have lowered blood pressure and blood glucose along with increased white blood cell and red blood cell parameters as a result of real acupuncture stimulation when compared to sham acupuncture.

MATERIALS AND METHODS

One hundred healthy privately-owned dogs, of various ages, breeds and sex were included in the study. The dogs were housed and fed consistent with the standard of care provided by their owners. Potential study dogs were given a physical exam consisting of assessment of body condition, posture, gait and behavior. After a thorough physical exam, a fasted blood sample was collected from dogs that were assessed as healthy. Dogs that were abnormal either on physical exam or blood work were excluded from the study. Once the dogs qualified for inclusion into the study, they were randomly assigned to either of 2 groups of 50 dogs by stratified randomization method.

The morning of collection of experimental data, the dogs were fasted. An oscillometric blood pressure measurement system[a] was used to measure the blood pressure of study animals. Cuff size used for pressure measurement was size 1 or 2 depending on the size of the dog and placed at the level of the median caudal artery on the forelimb.[33] The blood pressure of the dogs in the first group to be treated (Group A) was measured three times and the average value was then calculated. Blood was collected from the cephalica antebranchi vein and placed into a lithium heparin-containing tube for measurement of blood glucose levels by a commercial blood chemistry analyzer[b] or into an EDTA-containing tube for a complete blood count by a commercial hematology analyzer[c].

Bilateral acupuncture was then performed by a certified acupuncturist from Beijing Agricultural University at acupuncture points: Large Intestine-4 (LI-4), Stomach-36 (ST-36), Gall Bladder-34 (GB-34) and

Table 1: Anatomic descriptions of acupuncture points treated in study

Acupoint	Anatomic Location
LI-4	Located between the first and second metacarpal bones. Perpendicular insertion 0.5 cun deep.
ST-36	At the proximal one-fifth of craniolateral surface of the rear leg. Distal to the head of the tibia in a depression between the muscles of the cranial tibia and long digital extensor. Perpendicular insertion 0.5-1 cun deep.
GB-34	Cranial and ventral to the head of the fibula at the interosseous space. Proximal to the bifurcation of the superficial and deep peroneal nerves. Oblique insertion 0.5 cun deep.
SP-6	Located at the proximal end of the distal one-fifth of the tibia (from the medial condyle to the medial malleous), on the medial surface just caudal to the tibia. Perpendicular insertion 0.8-1.5 cun deep.

Figure 1: Localization of Large Intestine-4 (LI-4) acupoint in the dog. Medial view of left front leg.
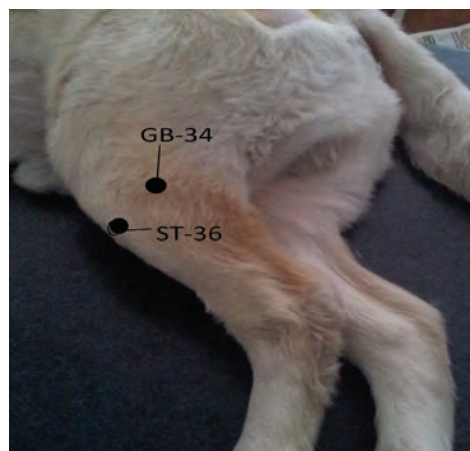
Figure 2: Localization of Stomach-36 (ST-36) and Gall Bladder-34 (GB-34) acupoints in the dog. Lateral view of left hind leg.

Figure 3: Localization of Spleen-6 (SP-6) acupoint in the dog. Medial view of left hind leg.

Spleen-6 (SP-6); (Table 1 and Figures 1-3). [5, 34-36] These acupoints were selected based on having previously been associated with modulation of blood pressure, blood glucose and/or hematology parameters.[8,12, 37-39] An acupuncture point detector[d] was used to determine the exact location of the acupoints. Stainless steel needles[e] measuring 0.20-0.25mm in diameter and 15-40mm in length were inserted and left in place for 20 minutes. Blood pressure measurements along with blood glucose and hematology values were repeated after the 20-minute acupuncture stimulation. The experimental parameters were measured again at one hour and finally at one week. After the one-week parameter measurement, acupuncture needles were then inserted into non-acupuncture points (sham acupuncture) and stimulated for 20 minutes. The measurements were repeated after 20 and 60 minutes (Table 2).

Group B received the reverse protocol. First, blood pressure, complete blood count (CBC) and blood glucose levels were measured and sham acupuncture was performed for 20 minutes. The measurements were then repeated after 60 minutes. After 7 days, the parameters were measured once again, and this time, acupuncture was performed at acupuncture points: LI-4, ST-36, GB-34 and SP-6 for 20 minutes. The blood pressure, blood glucose and hematology measurements (CBC) were repeated immediately and again 60 minutes after this period (Table 2).

The experimental repeated measures data collected on Group A and Group B in this crossover study was evaluated by one-way analysis of variance (ANOVA) method using commercial computer software[f] and Excel (Microsoft©). Statistical significance was set at $p<0.0001$, $p=0.0001$ and $p=0.0004$ level.

RESULTS

The age of the dogs in the first group (Group A) ranged between one and seven years (mean 2.79±1.53) with 28 intact males and 22 spayed females. The group included 25 crossbreeds, 8 Golden Retrievers, 7 Labradors, 2 Dachshunds, 2 Pugs, 2 Terriers, 2 Kangals, l German Shepherd, and 1 Samoyed dog. In Group B, the age of the dogs ranged between 1 and 5 years (mean 2.71± 1.31) with 21 spayed females and 29 intact males. This group included 25 crossbreeds, 4 Kangals, 4 Rottweilers, 4 Golden Retrievers, 3 Siberian Huskies, 5 German Shepherds, 3 Labradors, and 2 Yorkshire Terriers.

In both groups (A and B), diastolic and systolic blood pressure was reduced at 20 minutes following the real acupuncture period and although starting to increase after 60 minutes, it remained significantly lower compared to the pre-acupuncture level ($p<0.0001$) (Figures 4 and 5). During the sham acupuncture period a similar trend was observed in both groups but was so small, it did not attain statistical significance (Table 3). Blood glucose level following real acupuncture behaved in a similar manner in

Table 2: Experimental design where study animals crossed over from 1 treatment (real acupuncture points) to the other treatment (sham acupuncture points) during the trial.

| Experimental Procedure 1						
Groups	**Real Acupuncture**			**7 Days**	**Sham Acupuncture**	
	Baseline Pre-AP	20 minutes	60 minutes	Baseline Pre-AP	20 minutes	60 minutes
A	Hematology BL Pressure BL Glucose	Hematology BL Pressure BL Glucose	Hematology BL Pressure BL Glucose			
B				Hematology BL Pressure BL Glucose	Hematology BL Pressure BL Glucose	Hematology BL Pressure BL Glucose
Experimental Procedure 2						
Groups	**Real Acupuncture**			**7 Days**	**Sham Acupuncture**	
	Baseline Pre-AP	**20 minutes**	**60 minutes**	**Baseline Pre-AP**	**20 minutes**	**60 minutes**
A				Hematology BL Pressure BL Glucose	Hematology BL Pressure BL Glucose	Hematology BL Pressure BL Glucose
B	Hematology BL Pressure BL Glucose	Hematology BL Pressure BL Glucose	Hematology BL Pressure BL Glucose			

BL=Blood, AP=Acupuncture

Table 3: The mean values, standard deviations, and statistical significance of the data from Group A and B.

Experimental Procedure 1

Groups	Real Acupuncture		Pre- Acupuncture Mean/SD	20 minutes Mean/SD	60 minutes Mean/SD	*p*-value
Group A (n=50)	Systolic Blood Pressure		137.3±14.27	121.66±14.08	131.4±12.98	**P value <0.0001***
	Diastolic Blood Pressure		80.04±10.21	69.32±10.04	76.12±8.69	**P value <0.0001***
	Blood Glucose		114.41±14.24	103.09±13.26	105.52±13.44	**P value =0.0001***
	Hematocrit		37.77±4.55	37.13±3.83	37.60±3.57	P=0.7064 NS
	Hemoglobin		13.24± 1.8	13.32±1.73	13.28± 1.66	P value > 0.8 NS
	White Blood Cell	Male (n=28)	11.70±2.33	12.61±2.36	13.87±2.24	**P value <0.0001***
		Female (n=22)	9.42±1.74	10.53±1.48	11.76±1.32	**P value <0.0001***
Groups	Sham Acupuncture		Pre- Acupuncture Mean/SD	20 minutes Mean/SD	60 minutes Mean/SD	*p*-value
Group B (n=50)	Systolic Blood Pressure		138.2±13.41	136.14±13.80	136.12±12.35	P value=0.6653 NS
	Diastolic Blood Pressure		76.84±11.42	75.68±8.13	75.86±8.96	P value >0.8 NS
	Blood Glucose		119.47±11.84	115.91±9.89	119.33±9.99	P value= 0.1685 NS
	Hematocrit		38.46±2.57	38.1±3.64	38.36±2.69	P value >0.8 NS
	Hemoglobin		13.05±1.69	13.08±1.61	13.20±1.61	P value >0.8 NS
	White Blood Cell	Male (n=29)	12.49±2.15	12.49±1.86	12.71±1.91	P value >0.8 NS
		Female (n=21)	11.13±1.14	11.18±1.17	11.16±0.93	P value=0.9 NS

Experimental Procedure 2

Groups	Real Acupuncture		7 Days and Pre- Acupuncture Mean/SD	20 minutes Mean/SD	60 minutes Mean/SD	*p*-value
Group B (n=50)	Systolic Blood Pressure		136.9±10.76	127.06±8.76	131.84±9.33	**P value <0.0001***
	Diastolic Blood Pressure		76.92±9.07	67.82±7.10	73.32±6.65	**P value<0.0001***
	Blood Glucose		116.93±9.35	103.84±8.38	107.13±10.65	**P value<0.0001***
	Hematocrit		38.42±2.56	38.51±2.66	38.61±3.04	P value > 0.8 NS
	Hemoglobin		13.25±1.66	13.17±1.56	13.12±1.50	P value > 0.8 NS
	White Blood Cell	Male (n=29)	11.01±0.52	12.13±0.56	13.26±0.73	**P value <0.00001***
		Female (n=21)	9.4±1.78	10.5±1.51	11.73±1.33	**P value <0.0001***
Groups	Sham Acupuncture		7 Days and Pre- Acupuncture Mean/SD	20 minutes Mean/SD	60 minutes Mean/SD	*p*-value
Group A (n=50)	Systolic Blood Pressure		137.02±14.02	135.04±13.03	136.56±13.32	P value = 0.7444 NS
	Diastolic Blood Pressure		80.46±8.90	78.46±7.77	80.5±5.90	P value=0.3138 NS
	Blood Glucose		115.5±11.89	113.95±9.65	116.68±9.26	P value=0.4173 NS
	Hematocrit		37.71±2.70	37.59±3.70	37.69±3.63	P value > 0.8 NS
	Hemoglobin		13.31±1.66	13.38±1.63	13.28±1.63	P value > 0.8 NS
	White Blood Cell	Male (n=28)	13.22±2.08	13.08±1.97	13.08±1.96	P value = 0.9 NS
		Female (n=22)	11.11±1.12	11.12±1.16	11.09±0.96	P value = 0.9 NS

SD: Standard deviations, (*): significant, NS: non-significant
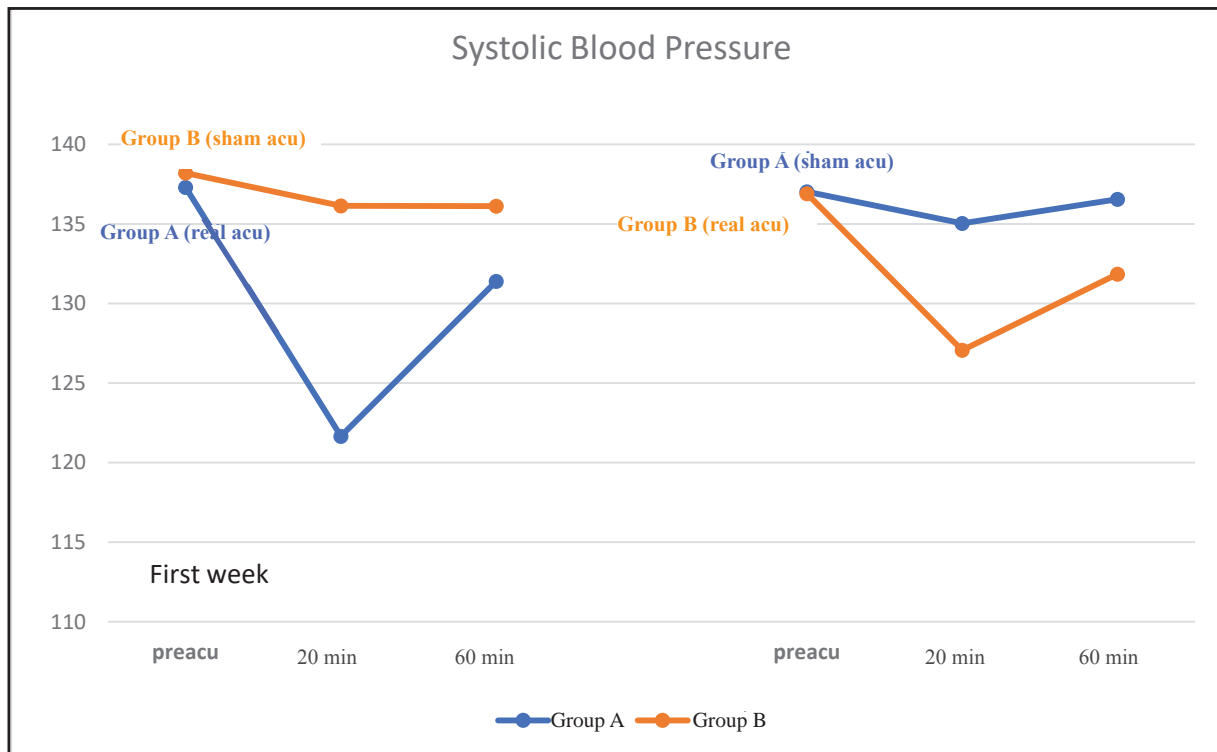
Figure 4: Comparison of systolic blood pressure of Group A (real acupuncture) and Group B (sham acupuncture) starting preacupuncture (preacu) and then at 20 minutes and 60 minutes after acupuncture during the first week. The comparison is demonstrated again after 7 days (preacupuncture measurement) followed by 20 and 60 minute measurements for Group A (sham acupuncture) and Group B (real acupuncture).
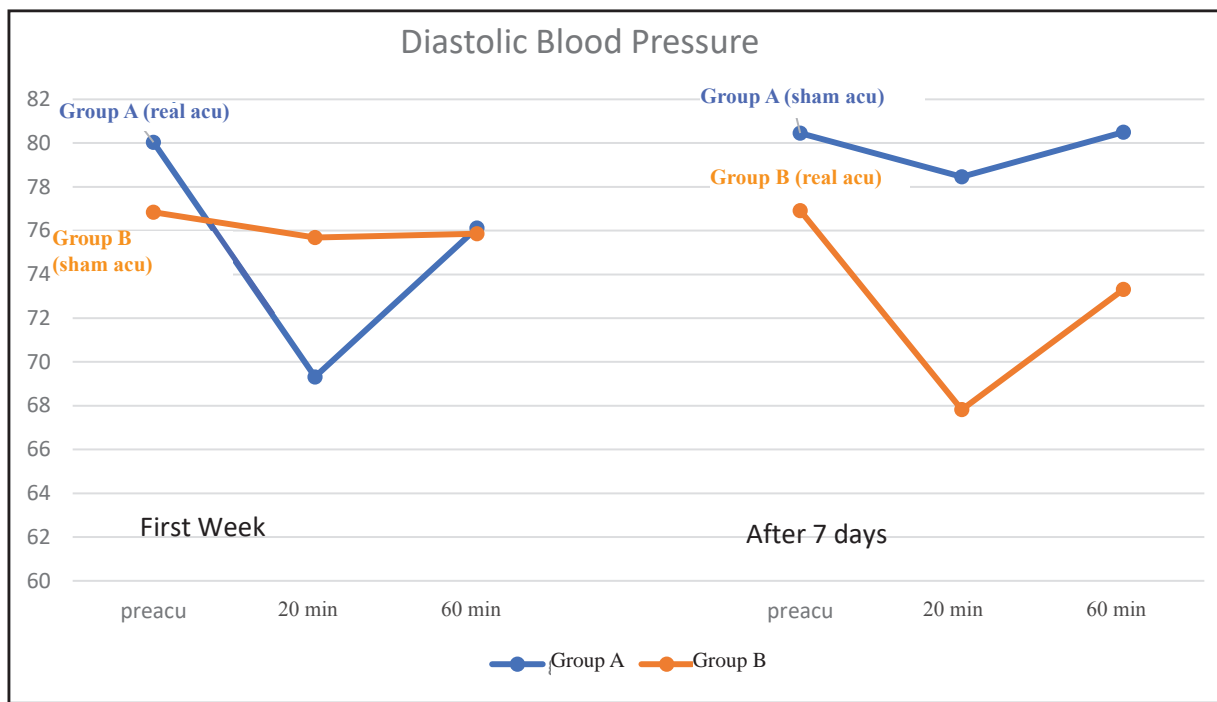
Figure 5: Comparison of diastolic blood pressure of Group A (real acupuncture) and Group B (sham acupuncture) starting preacupuncture (preacu) and then at 20 minutes and 60 minutes after acupuncture during the first week. The comparison is demonstrated again after 7 days (preacupuncture measurement) followed by 20 and 60 minute measurements for Group A (sham acupuncture) and Group B (real acupuncture).

both groups and decreased after 20 minutes compared to pre-acupuncture levels with an increase after 60 minutes but still remaining significantly lower than the initial level (p=0.0001). Sham acupuncture in both groups had a slight reduction in blood glucose levels at 20 minutes but returned to pre-acupuncture levels by 60 minutes (Figure 6).

The white blood cell count (WBC) was evaluated both with sexes combined and separated. When males and females were separated and results evaluated, there was a statistically significant increase in WBC values (*p* value <0.0001) in both sexes at both 20 minutes and 60 minutes after real acupuncture when compared to pre-acupuncture levels (Table 3). When male and female results were combined, there were variable decreases at 20 minutes with a statistically significant increase at 60 minutes. Values remained essentially the same with no significant changes observed during the sham acupuncture period. Evaluation of hematocrit (HCT) and hemoglobin (HGB) in the experimental groups, both when sexes were combined or separated did not have significant changes observed between real and sham acupuncture periods when comparing pre-acupuncture results to post-acupuncture (Table 3).

DISCUSSION

The results of this study demonstrated a statistically significant (p<0.0001) lowering of blood pressure when compared to baseline values in dogs at 20 and 60 minutes after 1 acupuncture session, which supported the study hypothesis for this parameter. The lack of change during the sham period in both groups demonstrated that the blood pressure change was not induced by other factors. It is thought that acupuncture reduces blood pressure by inhibiting the sympathetic nervous system, inducing the release of endogenous opiates, reducing the aldosterone level and inhibiting renin secretion. Recent studies have also recognized cellular effects such as modulation of medullary expression of microRNAs and increased nitric oxide as intimately involved in treating hypertension with acupuncture point stimulation. [8-10, 20-22, 37, 40-44] Similar to human studies, the author believes that the decrease in blood pressure following the acupuncture period, in both Group A and B, particularly in association with acupoint ST-36, induces inhibition of the sympathetic nervous system along with stimulating release of endogenous opiates. [22, 40, 41] The return to baseline values by Day 7 after 1 treatment suggests that for effective blood pressure control, acupuncture needs to be administered at repeated intervals (7-10 times) as suggested by other authors.[19-22, 40]

Blood glucose values had statistically significant decreases (*p*<0.0001) following the real acupuncture period in both groups at 20 minutes and although pressure was starting to rise after 60 minutes, this level was still significantly lower than the initial blood glucose reading. Similar to blood pressure results, the values were back to
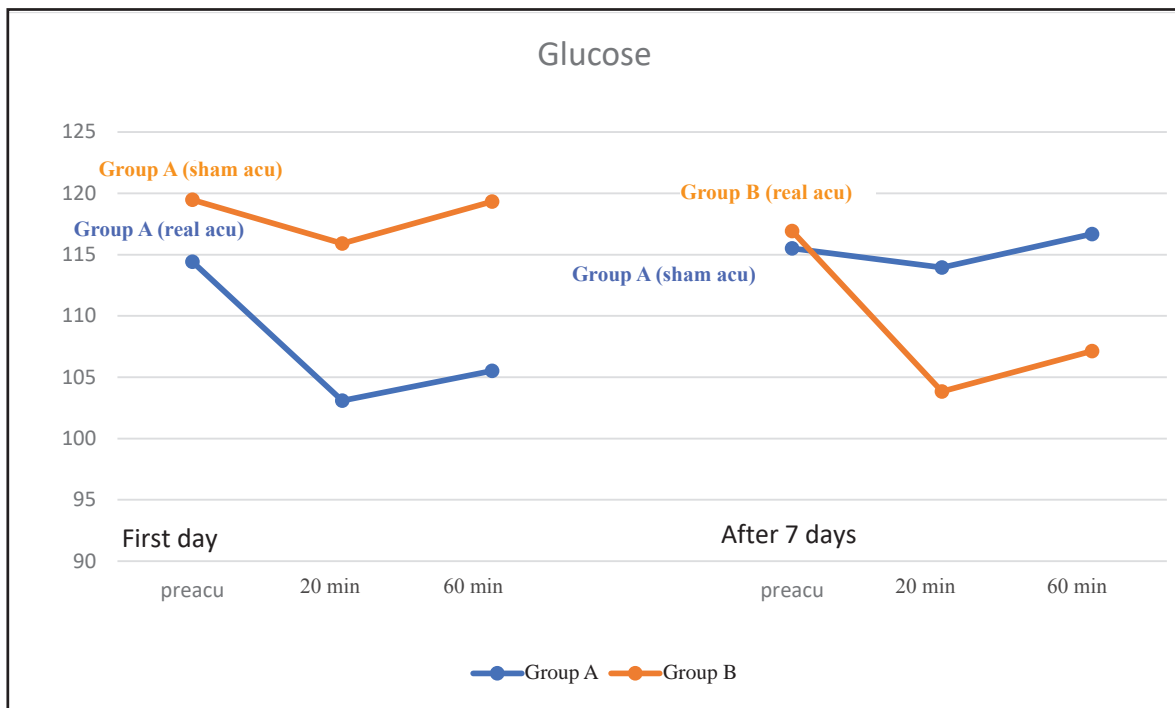
Figure 6: Comparison of blood glucose of Group A (real acupuncture) and Group B (sham acupuncture) starting preacupuncture (preacu) and then at 20 minutes and 60 minutes after acupuncture during the first week. The comparison is demonstrated again after 7 days (preacupuncture measurement) followed by 20 and 60 minute measurements for Group A (sham acupuncture) and Group B (real acupuncture).

baseline by Day 7 of the study suggesting that acupuncture to be effective must be incorporated into a treatment plan at regular intervals. As suggested by other authors in the literature, the significant reduction in blood glucose level at 20 minutes is associated with Spleen meridian effects.[23-25] The lack of such a change during the sham acupuncture period in both groups supports the hypothesis that the change in blood glucose level was not induced by other factors.

The antihyperglycemic effect of acupuncture, similar to acupuncture's effect on hypertension has been an active area of research both with preclinical studies and clinical trials in humans. In rodent studies acupuncture stimulation demonstrated significantly reduced blood glucose levels in obese model rats affected with type-2 diabetes as well as demonstrated prevention of diabetes when started in juvenile 6-week old rats. [28, 29] In clinical studies with obese type-2 diabetic human patients, acupuncture demonstrated clinical efficacy in lowering blood pressure, reducing body weight, decreasing blood glucose, lowering lipid and improving insulin resistance.[12-14, 17] Mechanistic studies with acupuncture have demonstrated the stimulation of endogenous beta-endorphin secretion which lowers blood glucose thus, being an excellent addition to therapy for glucose control in diabetic patients along with proper diet in an integrated medicine program.[23-27]

In this study, the WBC had statistically significant increases at 20 and 60 minutes when male and female results were evaluated separately in both real acupuncture groups but not when sexes were combined. Explanation for the need to separate sexes for this parameter most likely lies in the fact that spayed females and intact males were used instead of neutered males. If both sexes were agonadal, the hormonal profile would be similar. Intact males, however, have a testosterone effect, including levels of estrone sulfate the main metabolite of testosterone, whereas spayed females will have neither.

The lack of change for the WBC count, after stimulation of non-acupuncture points in both groups, indicates that no artefactual psychological or stress-induced effects were present. Similar to changes in this study, acupuncture increased white cells in the treatment of myelosuppression after chemotherapy and demonstrated improvement of neutropenia in gynecologic malignancies. In studies looking at immunomodulation with acupuncture treatment, activation of natural killer cell numbers and activity was demonstrated which enhances anticancer immune function.[9, 11, 45, 46]

Although some authors claim that acupuncture increases HCT and HGB levels, this change was not observed in this study population.[6] The male and female results were evaluated separately for these parameters to look for a testosterone effect which might increase red blood cell numbers of males, however, no statistically significant effects occurred. Individuals included in previous studies did not come from a healthy population.

Acupuncture normalizes hematology in unhealthy individuals, therefore, in the healthy population of dogs in this study, it is difficult to tease out any changes that may occur after the real acupuncture session in normal animals. This is one area that in future studies might yield better results by looking at an unhealthy population of dogs such as was done in human and preclinical studies.[38, 47]

In summary, the results of this study demonstrated that acupuncture at LI-4, ST-36, GB-34 and SP-6 was associated with statistically significant decreases in blood glucose, blood pressure and increases of WBC at 20 and 60 minutes with no adverse effects in a group of 100 healthy dogs. This study provides an evidence based support for acupuncture to be used as a beneficial therapeutic modality or supportive therapy in disorders affecting blood glucose such as diabetes mellitus or hypertension which may occur with multiple diseases without adverse effects. It also may be helpful in treatment of disorders of the immune system as suggested by the statistically significant increases in the white blood cell counts of study dogs.

ACKNOWLEDGEMENTS

FOOTNOTES
a. Vet HDO (high-definition oscillometry) Monitor :S+B medVet GmbH, Babenhausen, Germany.
b. VetTest Chemistry Analyzer 8008© ,IDEXX: West Brook, Maine, USA.
c. VetAutoread© Hematology Analyzer, IDEXX: West Brook, Maine, USA.
d. Pointer Pal, Tens Plus Industrial Company: HongKong, China.
e. Seirin Acupuncture Needles, Seirin Co.Ltd: Kyoto, Japan.
f. GraphPad Prism 7, Instat GraphPad Software, Inc.: La Jolla, California, USA.

REFERENCES
1. Brown M, Ulett G, Stern J. The effects of AP on white blood cell counts. AJCM 1974; (2):383-398.
2. Wu J, Hau D. Preliminary study on the effects of moxibustion on gamma-ray-irradiated mice. ARQ 1980; (4):91-105.
3. Hau D. Some haematological effects of moxibustion on X-ray irradiated guinea pigs. ARQ 1981; (5):7-18.
4. Rogers P. Immunological Effects of Acupuncture. In: Veterinary Acupuncture. Schoen A. (ed) St. Louis, MO: Mosby 1994: 243-268.

5. Klide A, Kung S. Veterinary Acupuncture. University of Pennsylvania Press. Third publishing. 1986: 297.

6. Stil J, Konrad J. The effect of acupuncture on hematologic and biochemical values in dogs with endoparasitic infections. Vet Med (Praha) 1985; 30(11) 687-698.

7. Lin J, Rogers P. AP effects on the body's defense systems: a veterinary review. Veterinary Bulletin 1980; 50:633-640.

8. Kim H, Cho S, Park S et al. Can acupuncture affect the circadian rhythm of blood pressure? A randomized, double-blind, controlled trial. J Altern Complement Med 2012;18(10):918-923.

9. Longhurst J, Tien-A-Looi S. Acupuncture regulation of blood pressure: two decades of research. Int Rev Neurobiol 2013; 111:257-271.

10. Cevik C, Iseri S. The effect of acupuncture on high blood pressure of patients using hypertensive drugs. Acupunct Electrother Res 2013;38(1-2):1-15.

11. Johnston M, Ortiz S, Vuianovic N et al. Acupuncture may stimulate anticancer immunity via activation of natural killer cells. Evid Based Complement Alternat Med 2011; edoc:article number 481625.

12. Peplow P, Baxter G. Electroacupuncture for control of blood glucose in diabetes: literature review. Acupunct Meridian Stud 2012; 5(1):1-10.

13. Mohanty S, Mooventhan A, Manjunath N. Effect of needling at CV-12 (Zhongwan) on blood glucose levels in healthy volunteers: a pilot randomized placebo controlled trial. Acupunct Meridian Stud 2016; 9(6):307-310.

14. Martinez B, Peplow P. Treatment of insulin resistance by acupuncture: a review of human and animal studies. Acupunct Med 2016; 34(4):310-319.

15. Fu H, Chen B, Hong S, et al. Acupuncture therapy for the treatment of myelosuppression after chemotherapy: A literature review over the past 10 years. J Acupunct Meridian Stud 2015;8(3):122-126.

16. Li P, Tien-A-Looi S, Cheng L et al. Long-lasting reduction of blood pressure by electroacupuncture in patients with hypertension: randomized controlled trial. Medical Acupuncture 2015;27(4):253-266.

17. Yang Y, Liu Y. BO's abdominal acupuncture for obese type-2 diabetes mellitus. Zhongguo Zhen Jiu 2015; 35(4):330-334. [Article in Chinese]

18. Brown S, Atkins C, Bagley R et al. Guidelines for the identification, evaluation and management of systemic hypertension in dogs and cats. ACVIM Consensus Statement, J Vet Intern Med 2007; 21(3):542-558.

19. Guo W, Ni G. The effects of acupuncture on blood pressure in different patients. J Tradit Chin Med 2003; 23(1):49-50.

20. Chiu Y, Chi A, Reid I. Cardiovascular and endocrine effects of acupuncture in hypertensive patients. Clin Exp Hypertens 1997; 19(7):1047-63.

21. Tam K, Yiu H. The effect of acupuncture on essential hypertension Am J Chin Med 1975; 3(4):369- 375.

22. Smith F. Acupuncture for Cardiovascular Disorders. In: Veterinary Acupuncture. Schoen A. (ed) St. Louis, MO: Mosby 1994:199-206.

23. Monnet, E. Effect of acupuncture on the glucose tolerance test in the dog. 13th Annual International Congress on Veterinary Acupuncture. Belgium.1987.

24. Ionescu-Tirgovist C. The hypoglycemic mechanism of the acupuncture point Spleen-6. Amer J of Acupuncture 1975;1:18-33.

25. Zeng Z, Li Y. Effects of electroacupuncture at Weiwanxiashu and Zusanli points on blood glucose and plasma pancreatic glucagon contents in diabetic rabbits. J Tradit Chin Med 2002; 22(2):134-6.

26. Chang S, Lin J, Chi T et al. An insulin-dependent hypoglycaemia induced by electroacupuncture at the Zhongwan (CV12) acupoint in diabetic rats. Diabetologia 1999; 42(2):250-255.

27. Chen D, Gong D, Zhai Y. Clinical and experimental studies in treating diabetes mellitus with acupuncture. Provincial Institute of TCM and Pharmacy. Jilin Province. PRC. JTCM 1994; (3):163-166.

28. Nakamura H, Ishigami T, Kawase Y et al. Effects of acupuncture stimulation on blood glucose concentration in the Otsuka Long-Evans Tokushima Fatty rat, an animal model for type-2 diabetes mellitus. Med Sci Monit Basic Res 2014; 20:70-75.

29. Cornejo-Garrido J, Becerril-Chavez F, Carlin-Vargas G et al. Antihyperglycaemic effect of laser acupuncture treatment at BL20 in diabetic rats. Acupunct Med 2014; 32(6): 486-494.

30. Lu M, Cao D, Zhao X. [Study on dynamic effect of acupuncture on marrow cell cycle regulatory protein cyclin D1 expression and cell cycle in mice with cyclophosphamide induced myelosuppression]. Zhongguo Zhong Xi Yi Jie He Za Zhi.2011; 31(2):238-243. [Article in Chinese]

31. Zhang K, Liu Z, Li G. Electro-acupuncture promotes the survival and differentiation of transplanted bone marrow mesenchymal stem cells pre-induced with neurotrophin-3 and retinoic acid in gelatin sponge scaffold after rat spinal cord transection. Stem Cell Rev 2014;10(4):612-625.

32. Lu W, Matulonis U, Doherty-Gilman A et al. Acupuncture for chemotherapy-induced neutropenia in patients with gynecologic malignancies: a pilot randomized, sham-controlled clinical trial. J Altern Complement Med 2009; 15(7):745-753.

33. Cunha A, Ramos S, Domingues M et al. Validation of noninvasive blood pressure equipment: which peripheral artery is best for comparison studies in dogs?. Veterinary Anesthesia and Analgesia 2017; http://dx.doi.org/10.1016/j.vaa2017.07.002.

34. Hwang Y. Canine Acupuncture Atlas. In: Veterinary Acupuncture. Schoen A. (ed) St. Louis, MO: Mosby

1994: 107-140.

35. Janssens L. Acupuncture points and meridians in the dog. The International Veterinary Acupuncture Society 1984.

36. Xie H, Preast V. Xie's Veterinary Acupuncture. Blackwell Publishing 2013; 359.

37. Severcan C, Cevik C, Acar H et al. The effects of acupuncture on the levels of blood pressure and nitric oxide in hypertensive patients. Acupunct Electrother Res 2012;37(4):263-275.

38. Xu M, Xu A, Sui M. Effect of lifting-thrusting reinforcing-reducing manipulations of acupuncture on serum ferritin content and total iron binding capacity in blood-deficiency syndrome rabbits. Changsha Municipal Hospital of Chinese Medicine, China; Zhen Ci Yan Jiu 2012;37(1):41-45.

39. An Y, Moon S, Min I et al. Changes in regional cerebral blood flow and glucose metabolism following electroacupuncture at LI 4 and LI 11 in normal volunteers. J Altern Complement Med 2009; 15(10):1075-1081.

40. Li P, Sun F, Zhang A. The effect of acupuncture on blood pressure: the interrelation of sympathetic activity and endogenous opioid peptides. Acupunct Electrother Res 1983; 8(1):45-56.

41. Lee M, Lee D, Kim S et al. Cardiovascular effects of acupuncture at Tsu San Li (St-36) in dogs. J Surg Res 1975; 18:51-63.

42. Anshelevich I, Merson M, Afanas'eva G. Serum aldosterone level in patients with hypertension during treatment by acupuncture. Ter Arkh 1985; 57(10):42-45.

43. Yang M, Yu Z, Deng S et al. A targeted metabolomics multiple reaction monitoring-mass spectromety (MRM-MS) study of identifying potential hypertension biomarkers in human plasma and evaluating acupuncture effects. Sci Rep 2016; 16 (6):published online, article number: 25871.

44. Wang J, Li H, Ma C. MicroRNA profiling response to acupuncture therapy in spontaneously hypertensive rats. Evid Based Complement Alternat Med 2015; Article number 204367.

45. Fu H, Chen B, Hong S et al. Acupuncture therapy for the treatment of myelosuppression after chemotherapy: a literature review over the past 10 years. J Acupunct Meridian Stud 2015; 8(3): 122-126.

46. Lu W, Matulonis U, Doherty-Gilman A. Acupuncture for chemotherapy-induced neutropenia in patients with gynecologic malignancies: a pilot randomized, sham-controlled clinical trial. J Altern Complement Med 2009 ; 15(7): 745–753.

47. Xie X, Cao Y, Gao Q et al. Acupuncture improves intestinal absorption of iron in iron-deficient obese patients: a randomized controlled preliminary trial. Chin Med J (Engl). 2017; 130(5): 508–515.