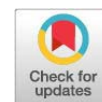


Research Article



Vibrational and Laser Acupuncture of Gv20 and Yintang as Sedative Adjuvant in Xylazine Sedated Dogs

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Abstract | This study aimed to evaluate laser and vibrational acupuncture at GV20 and Yintang as a sedative adjuvant in dogs. Five adult healthy mongrel dogs were randomly assigned to receive each of four sedation protocols at one-week interval. Hence, 4 groups were evaluated: XH (xylazine high: 1mg/kg of xylazine intravenously); XL (xylazine low: 0.25mg/kg of xylazine intravenously); XLL (xylazine low combined with laser acupuncture: 0.25mg/kg of xylazine intravenously with laser stimulation at GV20 and Yintang) and XLV (xylazine low combined with vibrational acupuncture: 0.25mg/kg of xylazine intravenously with vibrational stimulation at GV20 and Yintang). Measured variables were degree and duration of sedation, effect on physiological and some hematobiochemical parameters and incidence of vomiting. Sedation was significantly higher in XH than other groups at certain time points. Slightly higher sedation was evident in XLL and XLV than XL (greater potentiality in XLL). Sedation was longer in XH followed by XLL then XL and lastly XLV. Vomiting was observed only in XL. Milder alterations in cardiorespiratory parameters and rectal temperature were recorded in XL, XLL and XLV than XH. Significant changes in hematobiochemical parameters were only detected in erythrogram values and glucose concentrations in XLV and XH, respectively. On conclusion, despite of lower sedation in acupuncture groups than XH, laser acupuncture seemed to be effective in potentiating the sedative effect of low xylazine dose than vibrational acupuncture. Hence, laser acupuncture could be a promising sedative adjuvant in dogs sedated with low xylazine dose due to their poor tolerability to conventional high doses.

Keywords | Dogs, Laser acupuncture, Sedative, Vibrational, Xylazine

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INTRODUCTION

Xylazine is a potent α_2 -adrenergic agonist that induces sedation, analgesia, and muscle relaxation in dogs (Hazra et al., 2008). These characteristics can facilitate different diagnostic procedures and partly optimize canine general anesthesia. Virtually, α_2 -agonists are key factors in achieving smooth induction of anesthesia, reducing requirements of different anesthetics, and improving intraoperative stability (Tranquilli and Maze, 1993; Paddl-

eford and Harvey, 1999; Sinclair, 2003). Despite of this, administration of α_2 -agonists could be complicated with negative cardiovascular effects like bradycardia, cardiac arrhythmias, reduction in cardiac output, and an increase in systemic vascular resistance (Sinclair, 2003; Yaygingül and Belge, 2018). Reduction in breathing rate, hyperglycemia, decreased hematocrit, and vomiting are other adverse events (Ambrisko and Hikasa, 2002; Lemke, 2004; Santos et al., 2006; Baldo et al., 2012). In different animal species, the side effects of α_2 -agonists were described to

be dose-dependent (Wagner et al., 1991; Ambrisko and Hikasa, 2002). Hence, potentiation of the sedative effect of low doses of α_2 -agonists has been investigated to reduce the sedative doses of these agents and subsequently their drawbacks (Cassu et al., 2014). In this regard, the efficacy of pharmacopuncture (form of acupuncture) to potentiate the sedative effect of micro dose of xylazine (1/10 of intramuscular dose) has been evaluated in dogs (Cassu et al., 2014). Pharmacopuncture at Yintang acupoint potentiated the sedative effect of the micro dose of xylazine and induced lower adverse events compared to the conventional high dose (Cassu et al., 2014). Also, in dogs, pharmacopuncture with dexmedetomidine (α_2 -adrenergic receptor agonist drug) at another acupuncture point, GV20, produced greater and prolonged sedation than that achieved by identical doses given by intramuscular route (Pons et al., 2017). Subcutaneous administration of dexmedetomidine in dogs at GV20 also provided greater and faster sedation and similar recovery to intramuscular route (Leriquier et al., 2023). Stimulating GV20 and Yintang acupuncture points even via different acupuncture techniques as needle and electroacupuncture effectively induced sedation in conscious dogs (Kim et al., 2006; Kim and Seo, 2007).

The potential benefits of acupuncture at GV20 have been also proven for other pharmacological agents, opioids, whereas pharmacopuncture with low-dose hydromorphone at GV20 provided adequate postoperative analgesia in dogs undergoing ovariohysterectomy. Accordingly, pharmacopuncture at GV20 has been suggested as a good alternative in dogs when reduced dosing of opioids is recommended (Scallan et al., 2021).

Laser acupuncture and vibrational acupuncture are other acupuncture techniques. Laser acupuncture proved to be efficient in providing adequate postoperative analgesia in bitches submitted to ovariohysterectomy (Taffarel et al., 2013; Tomacheuski et al., 2020). Vibration therapy was also effective in stimulating some acupoints and treating headache in human (Elliot et al., 2019). Laser acupuncture and vibrational stimulation of acupoints could be considered as non-invasive modalities of acupuncture. Consequently, these techniques could be advantageous over invasive ones like needle, electroacupuncture and pharmacopuncture as eliminating patient discomfort and risk of infection (Gottschling et al., 2008; Litscher, 2009). From clinical point of view, laser acupuncture has been also described as an easier acupuncture method to apply relative to electroacupuncture (Nascimento et al., 2019).

Due to the previously mentioned merits of laser and vibrational acupuncture over pharmacopuncture and other invasive techniques as well as their efficacy in variety of conditions altogether with the clinical value of reduc-

ing the sedative doses of xylazine in dogs. This study aimed to investigate with the efficacy of laser and vibrational acupuncture as sedative adjuvant in xylazine sedated dogs. We hypothesized that these acupuncture modalities would effectively stimulate GV20 and Yintang acupuncture points and potentiate the sedative effect of low xylazine doses producing similar degree of sedation and lower adverse events compared to the conventional high dose.

MATERIALS AND METHODS

ANIMALS

Five adult healthy mongrel dogs (4 males and 1 female) weighing 14-18 kg and aged 1-4 years old were enrolled into this study. The studied dogs had a body condition score, 4-5 on scale from 1 to 9. Dogs were considered healthy after physical examination and routine hematological and serum biochemical analysis. The dogs were housed in individual kennels at Department of surgery, Anesthesiology & Radiology (Faculty of Veterinary Medicine, University of Sadat City). Dogs were allowed to acclimatize to their housing environment prior to the start of study. While the dogs were in their kennels, food and water were provided ad libitum however prior to experimental procedures, food and water were withheld for 12 and 3 hours, respectively. The present study was approved by the Institutional Animal Care and Use Committee of Faculty of Veterinary Medicine, University of Sadat City (protocol number: VUSC-047-1-22). Throughout the study, all efforts were exerted to avoid animal suffering.

EXPERIMENT DESIGN

This study was conducted as blinded randomized crossover study. In this experimental study, animals were randomly (Research Randomizer, Computer software, <http://www.randomizer.org/>) assigned to receive each of four sedation protocols (four groups were evaluated) with a week wash out period in between. For animal preparation, at the end of the day prior to experiment, the hair over the proposed acupoints (Yintang and GV20) (to facilitate placement of the electronic point finder and preserve blinding during evaluation of other protocols) was shaved. At the day of the experiment, animal were kept in experiment room for 30 minutes prior to assessment of protocols to acclimatize to the surrounding. The evaluated groups were as follows: Group 1 (XH: Xylazine high): Dogs received 1mg/kg of xylazine intravenously. Group 2 (XL: Xylazine low): Dogs received 0.25mg/kg of xylazine intravenously. Group 3 (XLL: Xylazine low combined with laser acupuncture): Dogs received 0.25mg/kg of xylazine intravenously. After 5 minutes, laser stimulation was conducted for GV20 acupoint followed by Yintang acupoint (each stimulated for 15 second), using a laser therapy device

(Cozing laser acupuncture pen, Wuhan Cozing Medical Devices Co., China) with the following parameters: 650 nm wavelength, output of 200 mW and a spot size of around 8 mm.

Group 4 (XLV: Xylazine low combined with vibrational acupuncture): Dogs received 0.25mg/kg of xylazine intravenously. After 5 minutes, vibrational stimulation was conducted for GV20 acupoint followed by Yintang acupoint (each stimulated for 15 seconds at 3600rpm) using rechargeable intelligence percussive message gun (MA 8816, Monlove, China).

The proposed acupoints were located as previously described, GV20, at the dorsal midline of the skull, at the rostral end of the external sagittal crest (Hwang and Limehouse, 2001) and Yintang, at midway between the medial ends of the two eyebrows (Kim and Seo, 2007) (Figure 1). Location of these acupoints was further verified by an electronic point finder (Silberbauer PS3 Point Locator, DI. Gerhard Silberbauer Hiessgasse, Wien, österreich) (Figure 2).

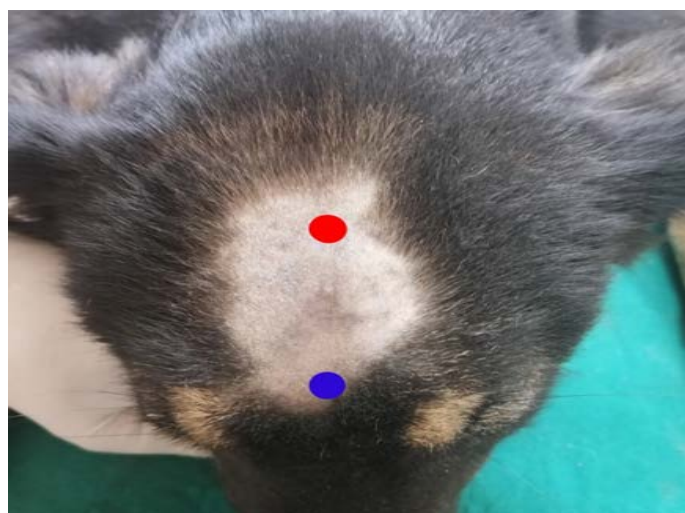


Figure 1: Location of GV20 (red circle) and Yintang (purple circle) acupoints in dogs.

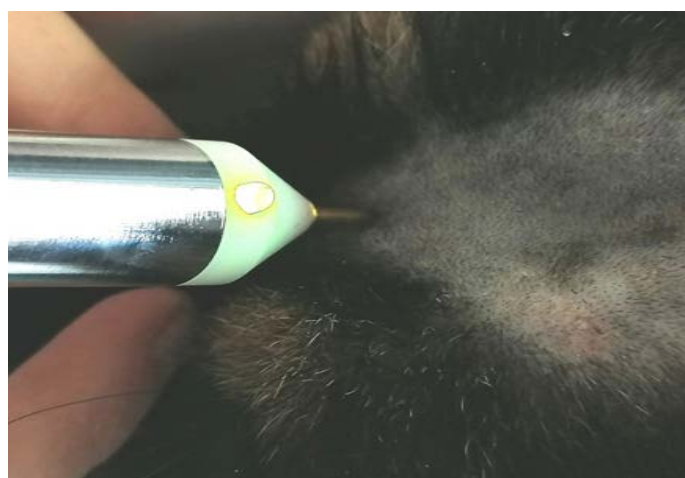


Figure 2: Using Silberbauer PS3 Point Locator for finding Yintang acupoint in one of the studied dogs.

ASSESSMENT METHODS

Degree and duration of sedation: Degree of sedation (represented as sedation score) was evaluated using a multidimensional sedation scale adapted from a previous report (St. James et al., 2019). This scale was dependent on categorization of spontaneous posture and determination of palpebral reflex, eye position, response to noise (hand-clap) and resistance of dogs when laid into sternal /lateral recumbency. A detailed description for these criteria is shown in Table 1.

According to the scoring system, a score of 0 was graded as no sedation, a score from 1-5 was graded as mild sedation, a score from 6-10 was graded as moderate sedation and a score from 11-15 was graded as intense sedation. Degree of sedation was assessed at baseline (prior to administration of xylazine) and after xylazine administration by 5,15,30,45,60,75 and 90 minutes by single blinded investigator (the same throughout the study). Duration of sedation was determined as the period over which sedation scores was significantly higher than baseline.

Effect on physiological parameters: Physiological parameters involving heart rate, respiratory rate, blood pressure and temperature were monitored at similar time points to sedation scoring. Heart rate (HR) was measured by auscultation with a stethoscope over 1 minute. Respiratory rate (RR) was monitored by counting the number of thoracic excursions also over one minute.

Non-invasive measurement of arterial pressure (systolic (SAP), diastolic (DAP) and mean (MAP)) was performed via oscillometric technique (Contec patient monitor, Contec medical systems Co, Qinhuangdao, China) with the cuff positioned at mid forearm (children non-invasive blood pressure (NIBP) cuff, 12-19 cm). Rectal temperature (RT) was measured in degrees Celsius (°C) using a digital thermometer (Double win company, China).

The incidence rate of vomiting: Vomiting episodes were recorded over the 90 minutes observation period.

Effect on hematobiochemical parameters: For evaluation of the hematobiochemical parameters, 2.5 ml venous blood sample was collected. About 1.0 ml was placed in EDTA tubes for complete blood count (red blood cells count (RBCs) count, hemoglobin (Hb) concentration packed cell volume (PCV) values, total leukocytic count (TLC) and differential leukocytic count). The rest 1.5ml of blood was placed in sodium fluoride tubes with subsequent centrifugation at 1600g for 14 minutes for separation of plasma. The harvested plasma samples were stored at -80°C for subsequent measurement of biochemical parameters, serum concentrations of total protein (TP), albumin, and

glucose using spectrophotometric method. Blood samples were collected at some time points (baseline and after xylazine administration by 5,15,30, 60 and 90 minutes).

Throughout the experiment, evaluation followed the same order (degree of sedation, physiological parameters, blood samples collection for hematobiochemical analysis).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 14.0 software (SPSS, USA). The measured variables were analyzed using a one-way analysis of variance (ANOVA) with Dunnett's post-test for comparisons within each group in relation to baseline. Comparisons between groups at each time were performed with one-way ANOVA followed by Tukey's test. The results of physiological and hematobiochemical parameters were expressed as mean $\pm$ standard deviation whereas the results of sedation degree (sedation scores) were expressed as median (range). The level of significance will be set at $p < 0.05$.

RESULTS

Based on sedation scores, at 8 minutes observation period, moderate degree of sedation was recorded in XH group (5/5 of the studied dogs) while in other treatment groups (XL, XLL and XLV groups) mild (4/5 of the studied dogs in XL and 3/5 in XLL and XLV) to moderate (1/5 of the

studied dogs in XL and 2/5 in XLL and XLV) degree of sedation was demonstrated. At 15 minutes, mild to moderate degree of sedation (1/5 and 4/5 of the studied dogs, respectively) was evident in XH group while in other treatment groups, only mild degree of sedation (5/5 of the studied dogs) was demonstrated. Over subsequent time points, degree of sedation tended to decrease gradually in all treatment groups with maintaining some differences among specific groups at certain time points.

Sedation scores (degree of sedation) were significantly higher in XH group compared to XL at 8 minutes observation period and compared to XL, XLL and XLV at 15 minutes. From 30 up to 60 minutes, sedation scores were significantly higher in XH group compared to XL and XLV groups. No significant difference was detected among treatment groups at 75 and 90 minutes. Throughout the entire observation period, sedation scores did not vary significantly among XL, XLL and XLV despite of this, scores tended to be slightly higher in XLL group relative to XL and XLV groups at most time points. At the first two observation periods, sedation scores tended also to be slightly higher in XLV group relative to XL group. With respect to duration of sedation (significant increase in sedation scores relative to baseline), sedation was evident for 60, 30,45,15 minutes in XH, XL,XLL and XLV groups, respectively.

Table 1: Multidimensional sedation scale for scoring sedation.

Variable	Score	Description
Posture	0	Standing position, normal standing posture
	1	Tired but standing, altered standing posture
	2	Lying, rising with ease when stimulated
	3	Lying, rising with difficulty when stimulated
	4	Unable to rise
Palpebral reflex	0	Brisk
	1	Slow but with full corneal sweep
	2	Slow but with partial corneal sweep
	3	Absent
Eye position	0	Central
	1	Rotated forward/downward but not obscured by third eyelid
	2	Rotated forward/downward and obscured by third eyelid
Response to noise	0	Normal startle reaction (head turn toward noise/cringe, head left, ear movement)
	1	Reduced startle reaction
	2	Minimal startle reaction
	3	Absent reaction
Resistance when laid into sternal / lateral recumbency	0	Some struggling or not allowing to be put into position
	1	Slightly reduced struggling
	2	Minimal struggling
	3	No struggling

Table 2: Median (range) of sedation scores and Mean $\pm$ SD values of physiological parameters in dogs after XH (xylazine high), XL (xylazine low), XLL (xylazine low combined with vibrational acupuncture) and XLV (xylazine low combined with laser acupuncture) treatments.

Time (minutes)									
Variables	Treatments	Baseline	8	15	30	45	60	75	90
Sedation scores	XH	0(0-0)	7(6-9)* ^δ	7(5-9)* ^α	3(3-9)* ^β	3(2-8)* ^β	3(2-4)* ^β	1(1-4)	1(0-2)
	XL	0(0-0)	4(2-6)*	2(1-3)*	1(1-3)*	1(0-1)	0(0-1)	0(0-0)	0(0-0)
	XLL	0(0-0)	5(4-9)*	3(2-5)*	2(2-3)*	2(1-3)*	2(0-3)	0(0-1)	0(0-0)
	XLV	0(0-0)	5(3-8)*	3(1-3)*	1(0-3)	1(0-1)	1(0-1)	1(0-1)	1(0-1)
RR (breaths/minute)	XH	17.80 $\pm$ 1.92	10.20 $\pm$ 2.59* ^δ	11.40 $\pm$ 3.85*	11.20 $\pm$ 3.03*	12.60 $\pm$ 2.19*	12.20 $\pm$ 1.92*	12.20 $\pm$ 2.49*	14.00 $\pm$ 1.87
	XL	16.60 $\pm$ 2.88	16.60 $\pm$ 1.67	16.00 $\pm$ 5.09	14.60 $\pm$ 3.58	15.00 $\pm$ 5.00	15.00 $\pm$ 4.243	15.20 $\pm$ 4.92	15.40 $\pm$ 4.56
	XLL	15.80 $\pm$ 1.92	14.00 $\pm$ 3.24	13.60 $\pm$ 3.71	13.80 $\pm$ 2.39	14.60 $\pm$ 2.07	13.80 $\pm$ 2.17	13.80 $\pm$ 2.17	15.00 $\pm$ 1.41
	XLV	15.40 $\pm$ 2.70	14.20 $\pm$ 4.76	14.20 $\pm$ 3.96	12.60 $\pm$ 3.21	13.60 $\pm$ 3.21	13.40 $\pm$ 2.51	14.80 $\pm$ 3.11	14.60 $\pm$ 3.97
HR (beats/minute)	XH	78.60 $\pm$ 12.84	49.60 $\pm$ 6.47* ^β	52.60 $\pm$ 8.05*	51.20 $\pm$ 6.49* ^δ	57.00 $\pm$ 7.81*	64.00 $\pm$ 13.47	63.00 $\pm$ 11.55	65.00 $\pm$ 11.59
	XL	75.60 $\pm$ 10.11	63.60 $\pm$ 7.54	63.80 $\pm$ 8.17	69.20 $\pm$ 9.88	67.40 $\pm$ 9.99	68.20 $\pm$ 9.63	75.20 $\pm$ 6.06	73.20 $\pm$ 6.69
	XLL	85.20 $\pm$ 12.64	59.20 $\pm$ 7.29*	62.60 $\pm$ 10.64	65.40 $\pm$ 13.05	68.20 $\pm$ 18.82	68.60 $\pm$ 13.97	70.20 $\pm$ 10.64	78.00 $\pm$ 14.97
	XLV	90.00 $\pm$ 10.84	69.40 $\pm$ 9.07	63.60 $\pm$ 10.97*	67.80 $\pm$ 8.76*	73.20 $\pm$ 9.91	78.80 $\pm$ 14.24	82.40 $\pm$ 16.67	80.40 $\pm$ 14.05
SAP (mmHg)	XH	128.75 $\pm$ 12.61	147.75 $\pm$ 7.80*	139.25 $\pm$ 7.80 ^δ	120.75 $\pm$ 10.63	100.25 $\pm$ 6.18*	107.50 $\pm$ 6.66*	100.25 $\pm$ 6.39*	106.25 $\pm$ 9.95*
	XL	132.50 $\pm$ 9.00	127.00 $\pm$ 8.08	110.50 $\pm$ 9.85*	116.75 $\pm$ 4.03	112.25 $\pm$ 9.98	114.00 $\pm$ 4.08	124.00 $\pm$ 17.56	122.00 $\pm$ 14.69
	XLL	128.50 $\pm$ 12.87	158.75 $\pm$ 3.15*	146.00 $\pm$ 7.62 ^δ	137.25 $\pm$ 4.86 [°]	143.50 $\pm$ 7.047 [°]	145.50 $\pm$ 7.05 [°]	145.50 $\pm$ 9.33 ^β	146.00 $\pm$ 6.68 ^{βδ}
	XLV	121.25 $\pm$ 10.11	129.25 $\pm$ 10.11	143.75 $\pm$ 9.11* ^δ	141.00 $\pm$ 4.89 [°]	145.00 $\pm$ 8.16* [°]	146.25 $\pm$ 9.39* [°]	136.00 $\pm$ 17.15 ^β	137.25 $\pm$ 9.39 ^β
DAP (mmHg)	XH	86.25 $\pm$ 7.50	90.25 $\pm$ 11.70	86.00 $\pm$ 11.34	72.00 $\pm$ 10.29	59.00 $\pm$ 2.58*	64.25 $\pm$ 7.46*	78.25 $\pm$ 13.55	85.00 $\pm$ 17.11
	XL	92.25 $\pm$ 4.86	90.00 $\pm$ 6.98	82.25 $\pm$ 7.18	79.00 $\pm$ 3.27	76.75 $\pm$ 9.03 ^β	80.25 $\pm$ 10.14	88.25 $\pm$ 11.95	86.25 $\pm$ 10.72
	XLL	90.00 $\pm$ 5.48	115.75 $\pm$ 6.55* ^α	119.50 $\pm$ 7.00* [°]	108.75 $\pm$ 8.85* [°]	111.50 $\pm$ 9.04* ^{βδ}	110.00 $\pm$ 8.68* [°]	102.50 $\pm$ 5.07 ^β	110.25 $\pm$ 8.42* ^β
	XLV	89.75 $\pm$ 2.87	97.00 $\pm$ 1.63	111.50 $\pm$ 2.08* [°]	111.00 $\pm$ 0.82* [°]	112.50 $\pm$ 2.08* ^{βδ}	114.00 $\pm$ 3.27* [°]	105.50 $\pm$ 9.39* ^β	107.00 $\pm$ 9.79*
MAP (mmHg)	XH	100.00 $\pm$ 8.68	108.75 $\pm$ 8.77	102.75 $\pm$ 7.68	88.00 $\pm$ 8.37	74.00 $\pm$ 2.45*	77.75 $\pm$ 6.02*	84.50 $\pm$ 19.67	91.50 $\pm$ 16.54
	XL	106.50 $\pm$ 7.19	103.25 $\pm$ 9.54	93.25 $\pm$ 8.54	92.75 $\pm$ 2.63	89.75 $\pm$ 8.26 ^β	92.75 $\pm$ 4.27 ^β	101.00 $\pm$ 12.99	99.25 $\pm$ 10.72
	XLL	102.25 $\pm$ 8.18	129.75 $\pm$ 8.54* ^α	128.00 $\pm$ 6.78* [°]	117.50 $\pm$ 7.19* [°]	121.25 $\pm$ 8.18* [°]	120.5 $\pm$ 7.68* [°]	116.25 $\pm$ 6.29 ^β	121.50 $\pm$ 7.77* ^β
	XLV	99.00 $\pm$ 4.89	107.75 $\pm$ 4.50	121.75 $\pm$ 4.50* [°]	120.25 $\pm$ 2.06* [°]	123.00 $\pm$ 4.08* [°]	124.50 $\pm$ 5.32* [°]	115.50 $\pm$ 11.85* ^β	117.25 $\pm$ 12.26* ^β

RT (°C)	XH	38.60± 0.12	38.34± 0.09	38.06± 0.19	37.94± 0.43	37.72± 0.58*	37.68± 0.52*	37.70± 0.44*	37.68± 0.58*
	XL	38.70±0.44	38.50± 0.44	38.38± 0.61	38.20± 0.68	38.48± 0.73	38.22± 0.66	38.26± 0.65	38.28± 0.72
	XLL	38.70± 0.42	38.40± 0.62	38.32± 0.66	38.34± 0.49	38.54± 0.71	38.32± 0.49	38.32± 0.47	38.60± 0.42
	XLV	38.34± 0.40	38.14± 0.49	38.10± 0.67	38.14± 0.69	38.16± 0.72	38.18± 0.75	38.34± 0.71	38.24± 0.59

*Significantly different (p<0.05) from baseline. ^δ Significantly different (p<0.05) from XL. ^α Significantly different (p<0.05) from other treatments. ^δ Significantly different (p<0.05) from XL and XLV. ^α Significantly different (p<0.05) from XH and XL. ^δ Significantly different (p<0.05) from XH.

Table 3: Mean ± SD values of hematological parameters in dogs after XH (xylazine high), XL (xylazine low), XLL (xylazine low combined with laser acupuncture) and XLV (xylazine low combined with vibrational acupuncture) treatments.

Variables	Treatments	Time (minutes)					
		Baseline	8	15	30	60	90
RBCs (×10 ⁶ /μL)	XH	5.71±0.58	5.93±0.61	5.76±0.58	5.50±0.59	5.46±0.58	5.52±0.54
	XL	5.61±0.48	5.5±0.48	5.43±0.51	5.23±0.42	5.35±0.45	5.24±0.48
	XLL	5.68±0.49	5.22±0.58	5.18±0.66	5.18±0.43	5.08±0.54	5.11±0.64
	XLV	5.51±0.09	5.23±0.17	5.10±0.29	5.08±0.19	4.93±0.263*	5.03±0.56
Hb (g/dL)	XH	14.50±0.47	14.86±0.98	14.34±0.56	13.92±0.76	13.68±1.04	13.92±0.88
	XL	14.48±0.62	14.18±0.81	14.04±0.89	13.66±0.77	13.68±0.93	13.82±1.02
	XLL	14.44±0.27	13.48±1.15	13.22±1.23	13.14±0.92	12.98±1.18	13.01±1.43
	XLV	14.52±0.23	13.78±0.42	13.40±0.78	13.46±1.12	13.02±0.73*	12.90±0.82*
PCV (%)	XH	37.36±1.14	38.24±2.27	37.24±1.53	35.48±1.76	35.32±1.81	35.71±1.91
	XL	38.78± 1.87	37.78± 1.74	37.12± 1.86	36.22± 2.00	37.02± 2.59	36.00± 1.98
	XLL	38.48±1.07	35.60±2.48	35.20±3.43	34.92±1.83	34.26±2.87	34.48±2.63
	XLV	38.66±1.71	36.54±1.05	35.54±2.58	35.56±2.70	34.10±1.39*	33.52±1.78*
TLC (×10 ³ /μL)	XH	10.08±2.17	11.96±3.63	11.82±3.27	11.52±2.15	10.56±2.94	10.68±2.99
	XL	10.92± 2.08	10.58± 2.63	10.54± 3.08	10.08± 1.14	10.38± 2.81	9.54± 1.75
	XLL	10.92± 2.23	10.60± 2.31	10.50± 2.24	10.50± 2.20	10.16± 1.91	10.04± 2.23
	XLV	10.54± 1.13	11.24± 0.67	10.86± 1.87	9.84± 1.51	9.40± 2.05	9.02± 1.99
Neutrophils (×10 ³ /μL)	XH	5.64±2.57	7.04±30.47	6.86±30.29	6.58±2.60	5.74±2.92	6.30±3.78
	XL	6.08±1.90	6.00±2.60	6.22±2.68	5.84±1.42	5.96±2.40	5.36±1.70
	XLL	7.04±1.88	6.54±1.90	6.82±1.91	6.62±1.83	6.58±1.95	6.32±1.79
	XLV	6.32± 1.36	6.68± 1.11	6.24± 1.65	6.08± 1.12	5.48± 1.54	5.40± 1.28
Lymphocytes (×10 ³ /μL)	XH	3.40± .86	3.24± 1.56	3.66± 1.12	3.72± 0.99	3.64± 0.93	3.12± 1.29
	XL	3.38± 0.79	3.32± 0.78	3.00± 1.23	3.10± 1.26	2.92± 1.05	3.20± 1.30
	XLL	2.98± 1.02	3.14± 0.69	2.74± 0.97	2.92± 0.73	3.36± 1.11	2.72±0.58
	XLV	3.14± 0.81	3.04± 0.54	3.40± 1.55	2.72± 1.42	2.86± 2.07	2.46± 1.32
Monocytes (×10 ³ /μL)	XH	1.04± 0.37	1.68± 1.25	1.30± 0.25	1.22± 0.23	1.18± 0.37	1.74± 0.93
	XL	1.46± 0.80	1.26± 0.57	1.30± 0.73	1.50± 0.74	1.50± 0.48	0.98± 0.34
	XLL	0.90± 0.19	0.92± 0.15	0.92± 0.16	0.96± 0.27	0.64± 0.11	1.00± 0.25
	XLV	1.08± 0.54	1.52± 0.49	1.20± 0.55	1.06± 0.46	1.06± 0.50	1.16± 0.49
Platelets (×10 ³ /μL)	XH	210.00±49.65	173.25±24.42	207.25±47.03	184.25±49.56	198.75±68.10	278.50±30.88
	XL	195.25±24.59	189.25±87.32	188.50±82.53	222.25±31.73	177.00±28.87	192.50±14.73
	XLL	206.50±41.13	246.25±20.42	237.75±27.80	247.75±23.08	218.25±60.17	255.75±30.18
	XLV	198.50±34.58	214.50±53.85	200.75±48.71	196.00±45.28	216.75±31.81	205.75±75.28

*Significantly different (p<0.05) from baseline.

For physiological parameters, relative to baseline, RR was significantly lower in XH group from 8 minutes up to 75 minutes without significant changes in other treatment

groups. Comparing groups, RR was only significantly lower in XH group compared to XL group at 8 minutes observation period. Significantly lower HR was detected in

XH group relative to baseline values from 8 minutes up to 45 minutes. Significant reduction in HR was also detected in XLL group at 8 minutes and in XLV group at 15 and 30 minutes. No significant changes were recorded in XL group. At 8 minutes, HR was also significantly lower in XH group compared XL and XLV groups while at 30 minutes, it was significantly lower in XH group compared to XL group only.

Relative to baseline values, SAP was significantly higher in XH group at 8 minutes while being significantly lower from 45 up to 90 minutes. SAP also tended to be significantly lower in XL group at 15 minutes while being significantly higher in XLL at 8 minutes and in XLV at 15,45 and 60 minutes. For DAP and MAP, compared to baseline, significant reduction was detected in XH group at 45 and 60 minutes while significant increment was evident in XLL at all-time points except at 75 minutes. In XLV group, DAP and MAP tended to be significantly higher than baseline values at all-time points except at 8 minutes. Throughout the observation period, significant differences in SAP, DAP and MAP were also detected among certain treatment groups.

For RT, it was significantly lower in XH group compared to baseline from 45 up to 90 minutes. No significant differences were detected in other treatment groups relative to baseline or in between groups at any observation period. Throughout the study, vomiting was only reported in XL groups whereas 2 of the studied dogs were vomited during early sedation period.

All data regarding sedation score and physiological parameters are listed in Table 2.

Regarding changes in the measured red blood cell parameters, compared to baseline, RBCs count was significantly lower in XLV group at 60 minutes while Hb concentration and PCV values were significantly lower at 60 and 90 minutes. No significant changes were detected in red blood cell parameters in other groups compared to baseline or among treatment groups at any recording time. For total leukocytic count, differential leukocytic count (neutrophil, lymphocyte and monocyte counts) and platelets count, no significant changes were detected in any group compared to baseline or between groups at any time point (Table 3). For biochemical parameters, no significant changes in TP and albumin levels were recorded in any treatment group compared to baseline or between groups throughout the observation period. Comparing with baseline values, blood glucose concentrations were significantly higher in XH group at 60 and 90 minutes without significant changes in other treatment groups. In between groups, blood glucose concentrations were significantly higher in XH group at 30

minutes relative to XLV and at 90 minutes relative to XL and XLL groups (Figures 3-5).

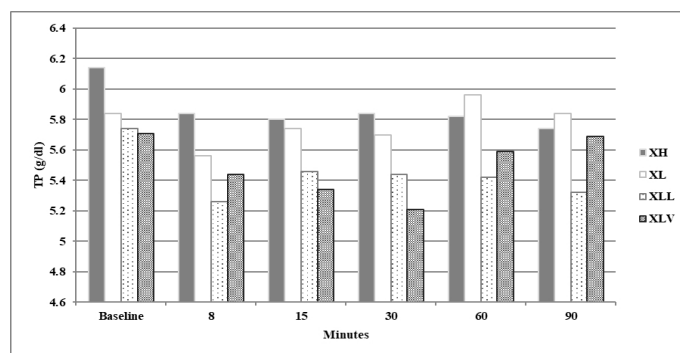


Figure 3: Mean values of TP (total protein) levels in dogs after XH (xylazine high), XL (xylazine low), XLL (xylazine low combined with vibrational acupuncture) and XLV (xylazine low combined with vibrational acupuncture) treatments.

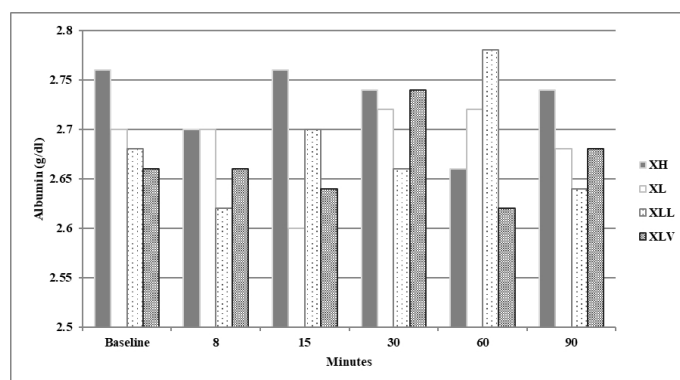


Figure 4: Mean values of albumin (total protein) levels in dogs after XH (xylazine high), XL (xylazine low), XLL (xylazine low combined with vibrational acupuncture) and XLV (xylazine low combined with vibrational acupuncture) treatments.

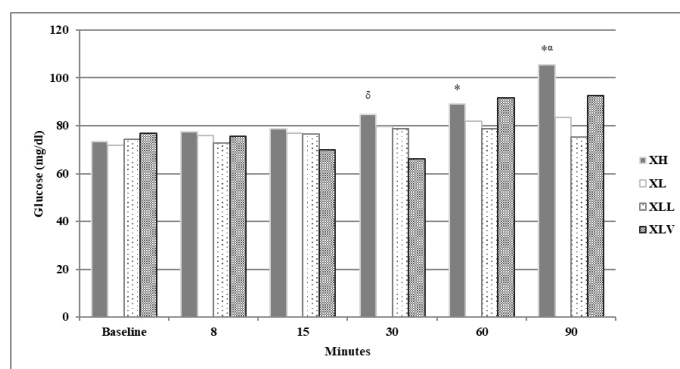


Figure 5: Mean values of blood glucose (total protein) concentrations in dogs after XH (xylazine high), XL (xylazine low), XLL (xylazine low combined with laser acupuncture) and XLV (xylazine low combined with laser acupuncture) treatments. *Significantly different (p<0.05) from baseline. δ Significantly different (p<0.05) from XLV. α Significantly different (p<0.05) from XL and XLL.

Among α_2 -adrenoreceptor agonists, xylazine was our drug of choice due to its availability and low cost which categorize it as the main sedative for dogs in Egypt. Further, xylazine is extensively used worldwide in dogs undergoing surgical and non-surgical procedures (Vesal et al., 2011; Silva et al., 2020; Lutvikadic and Maksimovic, 2022).

Under the conditions of the present study, following administration of different sedative protocols, sedation was evident in all groups (XH, XL, XLL and XLV). The resultant sedation in both XH and XL groups could be explained by the ability of α_2 -adrenoreceptor agonists to activate α_{2a} -receptors located in the locus coeruleus of the pons (Scheinin and Schwinn, 1992) with subsequent block of norepinephrine release (Sinclair, 2003). For XLL and XLV, the observed sedation could be explained in part by the previous assumption (as low xylazine dose was used also in both) along with sedation characteristics of GV20 and Yintang acupoints (Kim et al., 2006; Kim and Seo, 2007), mediated by activation of α_2 adrenoreceptors (Kim and Seo, 2007).

To assess degree of sedation in all treatment groups, a multidimensional sedation scale was elaborated. This scale seemed to be simple and successful as it effectively revealed the difference in the degree of sedation among groups. In this regard, sedation was significantly higher in XH group relative to XL, XLL and XLV groups at certain time points. This could be attributed to higher xylazine dose used in XH group relative to others.

The present study has addressed the sedative effect of 1mg/kg of xylazine (XH group), 0.25 mg/kg of xylazine (XL group), 0.25 mg/kg of xylazine combined with laser acupuncture (XLL) and 0.25 mg/kg of xylazine combined with vibrational acupuncture (XLV). Neither laser acupuncture nor vibrational acupuncture was assessed as a sole sedative protocol. This was conducted as the main target of the present study was to evaluate the potential benefits of these acupuncture modalities as sedative adjuvant not as sole sedatives. Further, in this study, no sham group (involves acupuncture at non-acupuncture points) was included as it was reported that sham acupuncture provides less profound effects compared to real acupoint stimulation (Sim et al., 2002).

It is well-established that application of an infrared laser with a wavelength between 650 and 1,000 nm has a penetration depth of 2 to 3 mm (Stolik et al., 2000), which can effectively stimulate acupoints (Litscher, 2009). Accordingly, in the current study, in XLL group, GV20 and Yintang acupoints have been stimulated with 650 nm wavelength laser to trigger effective stimulation of these points.

In related studies, the sedative effect of pharmacopuncture with micro doses of xylazine (Cassu et al., 2014) and acepromazine (Sousa, 2015) has been investigated and compared to conventional high doses. In these studies, 1/10 of the IM dose of xylazine (1mg/kg) and acepromazine (0.05 mg/kg) has been injected at Yintang point. Results revealed lower degree of sedation in pharmacopuncture group than that produced when high doses were administered IM despite of the potentiation of the drugs when given at the acupuncture point. For this, in the present study, in XLL and XLV groups, 1/4 rather than 1/10 of the conventional intravenous dose of xylazine (given in XH group) was given along with laser and vibrational acupuncture as an attempt to achieve comparable sedation to that produced by high xylazine dose in XH group. Further, in our study, laser and vibrational acupuncture was implemented at two acupuncture points, GV20 and Yintang rather than Yintang alone as in previous studies (Cassu et al., 2014; Sousa, 2015). This was selected as the clinical efficacy of acupuncture treatment has been greatly improved by synergistic and complementary effects produced by compatibility of different acupoints (Yang et al., 2021). Despite of this, even in the present study, lower sedation scores were recorded in XLL and XLV groups (at certain time points) compared to XH group. On these bases, to achieve comparable sedation, well designed studies are still required to optimize xylazine dose used in conjunction with acupuncture protocols. To upgrade acupuncture associated sedation, future studies could further investigate inclusion of another acupuncture points as PC6 besides GV20 and Yintang due to its anxiolytic properties (Schoen, 2003; Maccariello et al., 2018).

Despite of lower sedation in XLL and XLV groups relative to XH group, the ability of laser and vibrational acupuncture to potentiate the sedative effect of low xylazine dose couldn't be precluded. This could be evidenced by slightly higher sedation scores in XLL group relative to XL at most time points and in XLV group relative to XL group at the first two time points. This could further elucidate a greater potential of laser acupuncture than vibrational acupuncture at the used settings to potentiate xylazine related sedation. The greater efficacy of laser acupuncture could be also ascertained by prolonged sedation demonstrated in XLL group not in XLV group than XL group. The variable potency of these acupuncture modalities could be explained by the inherent characteristics of each acupuncture method (Sousa, 2015).

In XH group, significant decrease in RR was detected following administration of 1mg/kg of xylazine intravenously. In consistent with these findings, RR was significantly decreased when similar xylazine dose was given intramuscularly in dogs (Cassu et al., 2014). For XLL and XLV groups, RR did not vary significantly than baseline. On

contrary, in the study of Cassu et al. (2014), RR was significantly decreased after pharmacopuncture with smaller xylazine dose (0.1 mg/kg) than that (0.25 mg/kg) used in both acupuncture groups. This might be attributed to variable acupuncture modalities used in both studies whereas different acupuncture manipulations can generate distinct neural electrical codes (Yang et al., 2014).

Xylazine induces dose-dependent cardiovascular depression (Paddleford and Harvey, 1999). In the same way, contrary to XL group, significant reduction in HR was demonstrated in XH group with a greater reduction at certain time points. In XLL and XLV groups not in XL group, HR was significantly reduced at certain time points (one time in XLL and two points in XLV). This finding might be attributed to slightly higher sedation in these groups relative to XL group and the assumed ability of laser and vibrational acupuncture to induce some potentiation for the sedative effect of low xylazine dose. This could be supported by occurrence of α_2 -agonists associated bradycardia partly as result of decreased sympathetic outflow from the central nervous system (Sinclair, 2003) which related to the extent of resultant sedation.

In XH group, significant increase in blood pressure (SAP) was detected initially followed by prolonged decrease. In concordance with these findings, intravenous administration of xylazine in dogs at dose of 1 mg/kg has resulted in transitory increase in blood pressure (5–10 minutes) followed by a decrease in this variable to values lower than baseline values (Klide et al., 1975; Hsu et al., 1985). In XLL and XLV groups, arterial pressure (SAP, DAP and MAP) was significantly elevated than baseline values with significant elevation compared to XL and even to XH group at some time points. On the other hand, electroacupuncture at GV20 has reduced both systolic and diastolic blood pressure in hypertensive rats (Hwang et al., 2011). This discrepancy might be attributed in part to the difference in acupuncture techniques and animal models and their hemodynamic status. Another possible explanation for different results is stimulation of both GV20 and Yintang acupoints in the present study compared to GV20 alone in the other study (Hwang et al., 2011).

Contrary to XL, XLL and XLV groups, in XH group, significant reduction in RT was evident. Similar finding was reported when the same xylazine dose was administered intramuscularly in dogs (Cassu et al., 2014). Decreased heat production secondary to muscle relaxation or inhibition of thermoregulatory center could be the underlying mechanism for xylazine associated reduction in body temperature (Greene and Thurmon, 1988).

Vomiting has been reported following administration of xylazine to dogs (Paddleford and Harvey, 1999; Monteiro

et al, 2008). This was explained by the ability of α_2 -agonists to stimulate the chemoreceptor trigger zone, which is in close proximity to the locus coeruleus in the brain (Paddleford and Harvey, 1999; Tranquilli and Maze, 1993). In a previously related report, xylazine induced vomiting in 66% of dogs sedated with 1mg/kg of xylazine intramuscularly. In contrast to these findings, in the current study, no vomiting has been recorded in XH group following administration of 1mg/kg of xylazine intravenously. Different results might be attributable to different administration routes of xylazine which might predispose to specific effects. Also, in the present study, vomiting was only occurred in XL group. No vomiting was reported in XLL and XLV groups. This might support the ability of acupuncture to enhance the effect of subclinical doses of drugs altogether with reduction of their drawbacks (Luna et al., 2008).

No significant changes in red blood cell parameters were detected in XH group. On contrary, in a previous study by (Sutil et al., 2017), xylazine given at dose of 0.5 mg/kg IM has induced significant reduction in erythrogram variables. In XLV group, RBCs count and PCV values were significantly lower than baseline at certain time points. These results were not accorded by the results of (Kelawala et al., 1998) where electroacupuncture stimulation at GV-20 in combination with ST-36, SP-6, GV-6, BL-23, or TW 8, LU-1, GV-6 acupoints have induced significant increase in RBCs count and PCV values in dogs. This difference could be explained by stimulation of different acupoints besides GV20 in both studies as well as difference in acupuncture techniques.

Regarding changes in blood glucose concentrations, in XH group, glucose concentrations tended to be significantly higher compared to baseline and even to other treatment groups (XL, XLL and XLV) at certain time points. Consistently, dose-dependent increments in glucose values have been reported following xylazine administration to dogs (Ambrisko and Hikasa, 2002; Yaygingül and Belge, 2018).

CONCLUSION

Sedation was of greater degree and duration in XH group compared to XL, XLL and XLV groups. Slightly higher sedation was evident in XLL and XLV groups than XL group (more prominent in XLL group). Sedation lasted longer in XLL group than XL group. Accordingly, at the used settings, laser acupuncture at GV20 and Yintang acupoints was more effective than vibrational acupuncture to potentiate the sedative effect of low xylazine dose. Compared to XH group, milder effect on cardiorespiratory parameters and rectal temperature were recorded in XL, XLL and XLV groups. Significant changes in erythrogram val-

ues and glucose concentrations were detected in XLV and XH groups, respectively. Hence, laser acupuncture could be a promising sedative adjuvant in dogs sedated with low xylazine dose due to their poor tolerability to conventional high doses.

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NOVELTY STATEMENT

This study is the first study to investigate the efficacy of laser and vibrational acupuncture as sedative adjuvant in xylazine sedated dogs.

DATA AVAILABILITY

All data are included in this published article.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

FUNDING

The authors declare that no funds were received during the preparation of this manuscript

AUTHOR CONTRIBUTIONS

AH: Basic conceptualization of the project, data collection and sorting, interpretation of data and writing the primary version of the manuscript

AM: Data collection, interpretation of data and writing the primary version of the manuscript

SG & TM: Basic conceptualization of the project, interpretation of data and revise the manuscript.

All authors approved the final manuscript.

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