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Reversing Effect of Insulin on Analgesic and Motor-Blocking Action of Epidural Bupivacaine in Dogs

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Abstract

EPIDURAL anesthesia is a common technique in veterinary medicine despites lack of specific reversal agent for local anesthetics pose unique set of challenges. This study evaluated insulin as a reversal agent for epidural bupivacaine in dogs. Study involved two phases (phase 1&2). Phase 1: conducted on three dogs to determine the neurotoxic effect of epidurally administered insulin and to elaborate the effective reversal dose. Phase 2: involved six dogs receiving two epidural treatments at one-week interval. Two groups were assessed: BUP (epidural bupivacaine 1mg/kg + saline & saline infusion) and BUP/INS (epidural bupivacaine 1mg/kg + insulin 1IU/kg & dextrose infusion). Both groups were assessed for sedation, analgesia, motor blockade, plasma glucose and potassium concentrations, and serum bupivacaine concentrations. Phase 1 revealed no clinical neurological sequelae following epidural insulin and proposed 1IU/kg of insulin as a reversal dose. Phase 2 revealed comparable duration and degree of sedation in both groups. Analgesia and motor blockade durations were longer in BUP group than BUP/INS group. Significant changes in blood glucose concentrations were detected in both groups relative to baseline and even in between groups. Potassium concentrations significantly changed from baseline only in BUP group. Epidural insulin effectively reversed sensory and motor blocking action of epidural bupivacaine in dogs.

Keywords: Analgesic, Dogs, Epidural bupivacaine, Insulin, Motor-blocking action, Reversing effect**.**

Introduction

Epidural anesthesia is a frequently employed regional anesthetic technique in veterinary practice [1,2]. It is commonly used as a part of balanced anesthesia in dogs as it provides perioperative analgesia and muscle relaxation, reduces analgesic and general anesthetics requirements and reduces surgery associated stress response [3-9]. When combined with general anesthesia, epidural anesthesia promoted the quality of recovery and attenuated the immunosuppressive effects of anesthesia and surgery [7,10]. Epidural anesthesia can also alternate general anesthesia during several surgical procedures in small animal patients as cesarean section and hind limb orthopedic procedures [11-12].

Bupivacaine is an amide linked local anesthetic with prolonged analgesic and motor blocking action [13]. Considering analgesia, epidural administration of bupivacaine is commonly used to produce long-lasting caudal analgesia in dogs [14]. Meanwhile, prolonged motor block following epidurally administered bupivacaine [15], possibly contribute to increased post-anesthetic care unit recovery time and costs, owner dissatisfaction, patient anxiety and paralysis of hind limbs postoperatively [16,17].

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Local anesthetics have no specific reversal agent [18]. Hence, certain approaches and drugs have been investigated to reverse the prolonged effects of local anesthetics following different regional anesthetic techniques. Phentolamine mesylate (α - adrenergic antagonist/vasodilator) was one of the previously assessed drugs in human subjected to dental procedures. Phentolamine was effective to shorten the duration of action of different local anesthetics combined with epinephrine (α-adrenoceptor agonist/ vasoconstrictor) by enhancement of their systemic absorption [19]. However, local anesthetics without epinephrine couldn't be reversed in this manner [20].

With special respect to epidural anesthesia, in one study in dogs, epidural washout with saline has been addressed to reverse epidural anesthesia with lidocaine 2% with /without adrenaline. This approach sped up complete sensory and motor block recovery [21]. On the other hand, there was no consensus about the efficacy of this approach to hasten recovery from local anesthetics induced epidural block in human [22-24]. Insulin has been also assessed to reverse local anesthetics-induced effects. Insulin has been shown to be highly effective to reverse lidocaine and bupivacaine -induced sciatic nerve block in rat [20]. It was also effective at reversing the cardiac depressant effect of bupivacaine in dogs [25].

Despite of previously reported efficacy of insulin against bupivacaine -induced effects in animal models, its efficacy to reverse bupivacaine based epidural anesthesia has not been yet demonstrated. Hence, this study aimed to determine whether or not insulin would be able to reverse the motor block following epidural bupivacaine in dogs. Another objective was to determine if it would also reverse its analgesic effect. We hypothesized that insulin would reverse sensory and motor blocking action of epidural bupivacaine in dogs.

Material and Methods

Animals

This study was as a two-phased study (phase 1 and phase 2) carried out on nine adult healthy mongrel dogs (5 males and 4 females) weighing 14-22 kg and aged 1-3 years. Three of the studied dogs were enrolled into phase 1 (pilot study) while six mongrel dogs were assigned to phase 2. Dogs were considered healthy based on physical examination and routine blood analyses. Dogs were kept in separate kennels with unlimited access to food and water during the study period. Prior to experiment, food and water were withheld for 12 and 3 hours, respectively. This study followed ARRIVE guidelines on the use of experimental animals. Animals involved in the study were owned by the institution and used for teaching purposes. No euthanasia was conducted in relation to the study.

Experiment design

This experimental study was conducted in blinded randomized crossover design and involved two phases as follows:

Phase 1: this phase was a pilot study of two parts (part 1 and 2) to determine the neurotoxic effect of insulin (part 1) and to titrate insulin dose for elaborating the effective reversal dose (part 2). In this phase, the studied dogs (three dogs) were assigned to part 1 and 2 with an interval of one-week in between.

In part 1, to determine whether insulin has any neurotoxic effect, dogs were sedated with xylazine (Xyla-Ject®, 20 mg/ml, Adwia Co., Egypt) given intravenously at dose of 1 mg/kg. 5 minutes later, 1 IU/kg of insulin (short-acting insulin,100 IU/ml, Actrapid®, Novo Nordisk, Denmark) [25] adjusted to a total volume of 0.2 ml/kg using saline was injected into lumbosacral epidural space. At this time, dogs received an intravenous infusion of dextrose 50% (at a dose of 2 gm/1 IU insulin) [26] over 30 minutes. Following insulin injection and for 7 days later, dogs were monitored for any adverse events such as acute back pain (lumbar and sacral area) and tremors in lumbar muscles or hind limbs. The studied dogs were also observed for any abnormal behavior such as scratching, limping, restlessness and change in feeding pattern as well as dysfunctions (urinary and bowel dysfunctions) [27].

In part 2, sedated dogs (using xylazine similar to part 1) were randomly assigned to receive lumbosacral epidural administration of 0.2 ml/kg of 0.5% bupivacaine (a dose of 1mg/kg) (Sunnypuvacaine®, 100 mg/20 ml, Badr City, Egypt) followed after 15 minutes (from onset of action) either by a similar volume of saline or saline containing 1 IU/kg insulin or saline containing 0.5 IU /kg of insulin at one-week interval. For all these treatments, duration of sensory and motor block was determined.

Phase 2: in this phase, the studied dogs (six dogs) were randomly allocated to receive each of 2 treatments with a week wash out period in between. Hence, 2 groups were evaluated:

- Bupivacaine (BUP) group: 1 mg/kg of 0.5% bupivacaine at a volume 0.2 ml/kg administered into lumbosacral epidural space followed after 15 minutes (from onset of action) by a similar volume of saline.
- Bupivacaine/insulin (BUP/INS) group: 1 mg/kg of 0.5% bupivacaine at a volume 0.2 ml/kg administered into lumbosacral epidural space followed after 15 minutes (from onset of action) by a similar volume of saline containing 1 IU/kg insulin.

BUP/INS group received also an intravenous infusion of dextrose 50% (2 gm/1 IU insulin over 30 minutes) following epidural saline/insulin combination while BUP group received a similar rate

of saline infusion (to preserve blinding) for 30 minutes following epidural saline.

Just prior to sedating the studied dogs, each dog was kept in a quiet room to rest for 15 minutes. Later, in both groups, about 5 minutes after xylazine administration (same dose used in phase 1), cephalic vein was catheterized using a 20-gauge catheter (45 mm, Harsoria health care, Haryana, India).

The lumbosacral area was clipped and prepared for aseptic placement of an epidural needle. For localization of lumbosacral epidural space (L7-S1), dogs were placed in sternal recumbency with pelvic limbs drawn cranially [28]. A 22-gauge, 90mm spinal needle (Zhejiang Kindly Medical Devices Co., Zhejiang, PEOPLE'S REPUBLIC OF CHINA) was introduced into the epidural space at a 70–90° angle with the skin surface, with the bevel facing cranially [29] (Fig.1). Accurate needle placement was verified by hanging-drop method and loss of resistance to injecting treatments [30]. Prior to epidural injection, blood or cerebrospinal fluid was never aspirated into the epidural needle. In both groups, epidurals were injected slowly over 45 seconds while animals were in sternal recumbency. Animals were also kept in this position for 30 minutes later. All epidural injections were done by the same researcher.

Assessments

Degree and duration of sedation

Degree of sedation was qualitatively assessed using a simple descriptive scale (SDS) modified from [31] (Table 1). Sedation was evaluated at baseline, 5 minutes following xylazine administration and 15 minutes after bupivacaine onset of action up to 12 hours (every 15 minutes for 120 minutes and every 30 minutes later). Duration of sedation was the period over which mild to marked sedation was detected (sedation score >0).

Degree and duration of analgesia

Analgesia was assessed based on toe web pinch response (left hind limb) and skin pinch response (sacral and lumbar areas) [15,32].

For determining the toe web pinch response, standard nociceptive stimulus was applied to the left hind toe web of all four weight-bearing toes with a haemostatic forceps closed to the first ratchet. Based on animals' response, degree of analgesia in toe web was given scores ranging from 0-2 using SDS adapted from [15] (Table 2). Time to onset of complete analgesia, duration of complete analgesia and overall duration of analgesia were also recorded. Time to onset of complete analgesia was defined as time from drug administration to the appearance of complete analgesia (score = 2). Duration of complete analgesia was defined as time over which complete analgesia was observed (score = 2) while duration of analgesia was defined as time during which analgesia whether partial or complete was observed (score >0).

For skin pinch response, sacral and lumbar (till L1 dermatomes) areas were tested bilaterally in a caudocranial direction using skin pinching from Allis forceps. A positive response was considered as the reflex contraction of the skin [33]. Analgesia in these regions were assessed using a modification of SDS (scores 0-1) described by [15] (Table 2). Only complete analgesia could be evaluated in these areas because it was challenging to determine the intensity of skin contraction. Duration of analgesia in lumbar and sacral area dermatomes were recorded as time over which complete bilateral analgesia was demonstrated (score =1).

During evaluation of analgesia in the three body areas (left hind toe web / sacral and lumbar areas), to ensure that the animal's reaction was due to the painful stimulus applied (pinching toe web/sacral and lumbar areas) and not due to a learned behavior, non-nociceptive stimuli were applied when necessary by touching the tested areas.

Degree and duration of motor blockade

Degree of motor block was assessed using SDS (scores 0-2) adapted from [15] (Table 3). Time to onset of complete motor blockade, duration of complete motor blockade and duration of motor blockade were recorded. Time to onset of complete motor blockade was defined as time from drug administration to the time at which complete motor blockade appeared (score = 2). Duration of complete motor blockade was defined as time over which complete motor blockade was demonstrated (score = 2) while duration of motor blockade was defined as time during which partial or complete motor blockade was demonstrated (score >0).

Throughout the study period, evaluation of the sedation, analgesia as well as motor-blocking status was performed at the same time points by the same observer who was unaware of treatment given.

Effect on plasma glucose and potassium concentrations

For biochemical analysis, at certain time points (baseline, 5 minutes following xylazine administration and15,30,60,90,120,240,360,480,600 and 720 minutes after bupivacaine onset of action), about 1.0 ml venous blood sample was collected in sodium fluoride tubes with subsequent centrifugation at 2700 g for 10 minutes. The harvested plasma samples were stored at -80°C until plasma glucose and potassium (K^+) concentrations were measured using spectrophotometric method.

Effect on serum bupivacaine concentrations

For measurement of serum concentrations of bupivacaine, 3ml venous blood samples were

collected (at baseline and at 30, 60, 120, 240,480 and 720 minutes) in plain tubes with subsequent centrifugation at 2700 g for 10 minutes. Serum was harvested and stored at -80 °C until assayed. Bupivacaine was determined in serum samples by dispersive liquid- liquid microextraction method adapted from [34] using acetonitrile as disperser and decanol as an extractant with subsequent high performance liquid chromatography analysis (HPLC: Dionex UltiMate 3000 HPLC, Thermo Scientific™, Dionex™, Sunnyvale, CA, USA) with diode array detector. For HPLC, calibration samples were prepared to have bupivacaine concentrations of 0.1 (the limit of quantitation), 0.2, 0.4, 0.8, 1.2 ,1.6 and 2 µg/mL in dog serum.

During the entire experiment, measurement of the studied variables was conducted in the following order, evaluation of sedation followed by evaluation of analgesic and motor-blocking effects and lastly blood samples collection. Dogs were returned to their kennel when normal ambulation was observed.

Statistical analysis

For statistical power, sample size calculation was conducted using Andrew Fisher's formula. A study size of 6 dogs (for phase 2) was suggested to achieve a confidence level of 80%. SPSS 16.0 software (SPSS, USA) was used for statistical analysis. Non-parametric data (sedation, analgesia and motor blockade scores) were compared within each group relative to baseline and between groups at each time point using Wilcoxon signed-rank test. Parametric data (blood glucose concentrations and K^+ levels and serum bupivacaine concentrations) were compared within each group relative to baseline and between groups using paired samples t-test. A P value < 0.05 was regarded statistically significant.

Results

In this two-phased study, the resultant sedation following xylazine administration seemed to be sufficient for successful epidural punctures and injection of the tested medicaments. During epidural injection of different medicaments, only a slight body cringe was noted in the studied dogs.

In the pilot study (part 1), following epidural administration of insulin, no neurological sequelae were noted in any of the studied dogs. Also, in the pilot study (part 2), sensory and motor blockade durations following epidural bupivacaine were considerably reduced when dogs received a subsequent epidural injection of saline containing 1 IU/kg of insulin compared to saline containing 0.5 IU/kg of insulin or saline alone (data not shown). Also, in phase 2, in saline treated dogs, complete recovery from motor blockade was recorded in one of the three studied dogs only 12 hours after epidural administration of bupivacaine.

In phase 2, assessment of sedation revealed significantly higher sedation scores (degree of sedation) in BUP and BUP/INS groups relative to baseline for 60 and 45 minutes, respectively. In between groups, significant differences in sedation scores were not detected at any time point (over an observation period of 720 minutes). The overall duration of sedation was about 78 ±26.83 (mean ± SD) minutes in BUP group and 75±21.21 (mean ± SD) minutes in BUP/INS group.

Based on the toe web pinch response, in both BUP and BUP/INS groups, 5 minutes after xylazine administration, partial analgesia was developed in the left hind toe web of all studied dogs (n=6). Following epidural administration of bupivacaine, partial analgesia was progressed into complete analgesia in all dogs. The time to onset of complete analgesia was comparable in both groups: 6.2±2.2 (mean ± SD) minutes in BUP group and 6±0.71 (mean ± SD) minutes in BUP/INS group.

Throughout observation period, analgesia scores were significantly higher relative to baseline for 150 minutes in BUP group and for 120 minutes in BUP/INS group without significant difference among these groups. Despite of this, the duration of complete analgesia and the overall duration of analgesia were shorter in BUP/INS group (99±69.23 and 198.86.43 minutes, respectively: mean ± SD) compared to BUP group (135 ±83.52 and 252±78.23 minutes, respectively: mean ± SD).

Based on skin pinch response, complete analgesia in lumbar and sacral areas was evident 5 minutes after xylazine administration and continued after epidural administration of bupivacaine for variable durations in the studied groups. Complete analgesia in lumbar and sacral areas was of shorter duration in BUP/INS group (30±21.21 and 78±34.21 minutes, respectively: mean ± SD) relative to BUP group (39±17.10 and 99±37.65 minutes, respectively: mean ± SD). For statistical difference in analgesia scores, relative to baseline, significantly higher scores were detected in BUP group for 30 and 90 minutes in lumbar and sacral areas, respectively. While, in BUP/INS groups, significantly higher scores were detected for 15 and 60 minutes in lumbar and sacral areas, respectively. Comparing groups, no significant differences were demonstrated throughout observation period.

All data regarding sedation scoring and analgesia in toe web, lumbar and sacral areas dermatomes are listed in Table 4.

For motor blocking status, following xylazine administration, partial motor blockade was observed in all studied dogs of BUP and BUP/INS groups. With epidural administration of bupivacaine, complete motor blockade was exhibited by the studied animals of both groups. The time to onset of

complete motor blockade was 8.2±2.6 (mean ± SD) minutes in BUP group and 7.2±2.2 (mean ± SD) minutes in BUP/INS group.

Motor blockade scores persisted to be significantly higher relative to baseline for 240 minutes in BUP group and 180 minutes in BUP/INS group. Significantly higher scores were also demonstrated in BUP group compared to BUP/INS group at 60 minutes. Collectively, the duration of complete motor blockade and overall motor blockade was shorter in BUP/INS group (54 ±20.12 and 348 ±169.17 minutes, respectively: mean ± SD) compared to BUP group (99 ±65.04 and 510 ±246.48 minutes, respectively: mean ± SD). All data regarding motor blockade are depicted in Fig. 2.

Regarding blood glucose concentrations, in BUP group, concentrations were significantly higher from 15 minutes up to 120 minutes relative to baseline. In BUP/INS group, glucose concentrations were significantly higher than baseline at 15,30 and 60 minutes, but significantly lower from 90 minutes up to 360 minutes. Comparing groups, glucose concentrations were significantly higher in BUP/INS group than BUP group at 30 minutes, but significantly lower from 90 up to 360 minutes (Fig. 3). For blood K^+ levels, significantly higher levels were recorded in BUP group compared to baseline from 30 to 120 minutes without significant changes in BUP/INS group relative to baseline. Among groups, blood K^+ levels were significantly higher in BUP group than BUP/INS group at 90 and 120 minutes (Fig. 4).

Following epidural administration of bupivacaine, the peak serum concentrations were attained earlier in BUP/INS group (0.29 Mg/ml at 60 minutes) compared to BUP group (0.28 Mg/ml at 120 minutes). For both groups, the bupivacaine serum concentrations were significantly higher than baseline from 30 up to 720 minutes recording time with being significantly higher in BUP/INS group than BUP group at 30 and 60 minutes (Fig. 5).

Discussion

The advocacy of proper anesthesia management has raised great concern regarding reversal of the prolonged blocking- actions of local anesthetics. This was challenged by absence of specific reversal agent for local anesthetics [18]. Indeed, elaborating successful medicaments and approaches to reverse local anesthetics induced effects became a necessity. In veterinary studies, insulin and saline were assessed as potential reversal agents for local anesthetics [20-21]**.** Epidural washout with saline was effective to speed the recovery from epidural anesthesia with lidocaine in dogs [21]. Insulin was also effective to speed recovery from peripheral nerve block with lidocaine or bupivacaine in rats [20]. Based on the results of the fore mentioned studies, insulin seemed to be more effective as reversal agent (faster recovery from the blocking action of local anesthetics). Accordingly, this study focused on evaluating the ability of insulin to reverse motor and sensory block following epidurally administered bupivacaine in dogs.

In the previous report [20], complete recovery time of nerve (sciatic) conduction status was abbreviated when insulin was instilled over the exposed sciatic nerve. This could substantially denote the benign effect of insulin on peripheral neuronal tissues. Despites, as non-preservative free insulin (Actrapid) was selected to be assessed in the present study and as preservative-free solutions are recommended to epidural injections to avert the toxic effects of preservatives on the spinal cord [1], it was crucial to investigate the neurotoxic effect of insulin. For this, in the study reported here, insulin was administered epidurally in three pilot dogs that observed thereafter for any adverse event over 7 days [27]. This duration was selected to eliminate the potential that some neurotoxic effects could be masked by xylazine associated sedation and to provide a considerable time frame for any possible adverse event to be evident.

Further, to improve reliability of the findings of the assessment of insulin neurotoxicity, insulin treated dogs received an intravenous infusion of dextrose. This was attempted to ameliorate drastic reduction in blood glucose concentration and its possible negative impacts (such as seizures) [35] that might be misinterpreted as neurotoxicity if epidural insulin was released into systemic circulation and achieved a blood concentration sufficient to induce severe hypoglycemia whereas insulin basically lower blood glucose values [36].

Inadvertent epidural administration of insulin was previously recorded in human [37]. In this case report, no neurological sequelae were noted when insulin, a preparation containing preservatives was accidentally infused into epidural space. Consistently, in the present study, none of the studied animals showed any clinical neurotoxic signs when preservative containing insulin was injected epidurally. No neurotoxicity was demonstrated following an epidural injection of morphine with 0.1% sodium metabisulfite as preservative in dogs [27]. Intrathecal administration of neostigmine containing methyl- and propylparaben as preservatives in rats and sheep didn't further induce any histologic evidence of neurotoxicity [38]. Collectively, these findings can support the results of the current study.

As insulin did not produce any clinical abnormal behavior, two doses of insulin (1 IU/kg and 0.5 IU/kg) were subsequently evaluated against bupivacaine induced sensory and motor blockade in three studied dogs. This titrating study was attempted

to elaborate the effective reversal dose. The dose of 1 IU/kg was extrapolated from a previous study in dogs, reporting its efficacy in reversing bupivacaine-induced cardiac depression [25]. 0.5 IU/kg of insulin was also assessed as a nearly similar dose (0.4 IU/kg) was effective in reversing local anesthetic associated peripheral nerve block in rats [20].

Based on the pilot study (part 2 of phase 1), 1 IU/kg but not 0.5 IU/kg provoked a meaningful reduction in bupivacaine's sensory and motor blocking action in the three dogs under the study. Hence, 1 IU/kg was the dose enrolled into phase 2 to deeply investigate its effects.

Generally, in the study reported here (part 2 of phase 1& phase 2), epidural blocks following 0.2 ml/kg of 0.5% bupivacaine (a dose of 1mg/kg) was investigated. This volume was selected as it is often recommended for epidural anesthesia in dogs [6, 39]. The tested dose of bupivacaine, 1mg/kg was also chosen as it is repeatedly assessed experimentally and used clinically for multimodal analgesia in dogs [15,40]. In the previous study [15], bupivacaine given epidurally at a dose of 1mg/kg notably produced longer motor blockade compared to 0.5 mg/kg which was another determinant in dose selection.

In phase 2, sedation was scored and compared among BUP and BUP/INS groups to determine whether or not insulin given epidurally would modulate the intensity of sedation in the studied dogs. This was relied on insulin associated reduction in blood glucose concentrations [36] and hypoglycemic decreased alertness [35]**.** In the present study, the degree of sedation did not vary significantly between BUP and BUP/INS groups with slightly prolonged significance from baseline in BUP group. These findings might rule out any modulatory effect of epidural insulin at the used doses on sedation scoring. This would be valuable to avoid prolonged recovery if epidural insulin was used following general anesthesia in dogs to antagonize bupivacaine epidural anesthesia.

Epidural anesthesia stands for the sensory, motor and autonomic blockade resulting from epidural administration of local anesthetics [1]. Analgesia and motor-blocking action per se were the main focus of the present study. In this study, epidural administration of bupivacaine was carried out in xylazine sedated dogs. Considering the analgesic and the muscle relaxant effect of xylazine [41], complete analgesia and complete motor blockade was used to define the onset of bupivacaine epidural anesthesia.

In the study reported here, the onset of blockade (sensory/motor blockade) was about 6-8 minutes in BUP and BUP/INS groups. This was almost similar to that previously documented for bupivacaine in dogs [15, 42]. In the studied dogs, complete sensory block observed following epidural injection of bupivacaine could be supported by the unique ability of local anesthetic drugs to completely block transmission of nociceptive impulses or pain [43].

In BUP and BUP/INS groups, the overall duration of motor blockade produced by epidural bupivacaine was notably longer than that of the concomitant analgesia (toe web analgesia). Similar findings were previously reported in dogs when similar dose/volume of epidurally administered bupivacaine was evaluated [15]. In both studies, epidural bupivacaine did not provide differential blocks (sensory block being more prevalent than motor block). These findings might be attributed to the potential of bupivacaine to produce differential block at low doses/concentrations (<0.25%) rather than at high doses and concentrations (like the dose and concentration used here) [44]. Despite the overall duration of motor blockade was longer than analgesia in the present study, complete analgesia lasted longer than complete motor blockade. This variability might be due to the end point of motor block (normal motor response: normal ability to walk or stand using the hind limb) compared to sensory block (normal response: pelvic limb withdrawal and/or vocalization) that probably necessitate a greater recovery from local anesthetic induced block to be evident.

Analgesia and motor blockade were of shorter duration in BUP/INS group than BUP group. This could denote the ability of insulin given epidurally to reverse bupivacaine induced epidural blocks. This is in keeping with findings of the previous study in rats reporting the effectiveness of insulin to reverse peripheral nerve block with either lidocaine or bupivacaine [20]. The antagonistic mechanism of insulin against local anesthetics' induced block was previously explained [20] by its ability to enhance transient increase in outward K^+ current and repolarization [45] and in Ca2+ current [46] that are substantially inhibited by local anesthetics [47-48]. Despite of the consistency between our study and previous study [20], the relative ability of insulin to reverse bupivacaine induced block was more prominent in the later study (68.9% reduction in block duration) compared to the current study (21.4% and 31.8% reduction in sensory and motor block duration, respectively). This might be attributed to greater blood uptake (large no of epidural blood vessels) and larger no of the concerned nerves following epidural injection [49] compared to perineural injection which probably decreased insulin concentration at the nerves in situ.

In the previous study [21], epidural washout with saline after epidural anesthesia, speed up the regression of lidocaine induced motor and sensory block. In the present study, saline was used as a vehicle for insulin. Despite of the smaller volume used in the present study (0.2 ml/kg, maximally 4.4 ml) compared to the previous study (10 ml) [21] that

is not expected to produce a meaningful wash out effect, a similar volume of saline was injected epidurally in BUP and BUP/INS groups (containing insulin in BUP/INS) to ensure that the obtained results are related to insulin itself.

Based on the present study, motor block duration was greatly reduced by injection of insulin compared to analgesia (31.8% versus 21.4%). This might be due to different kinds of K channels (the target of insulin) in motor and sensory nerve fibers [45, 50]. Presumably, motor fibers channels are more sensitive to insulin which promoted the resultant antagonistic effect. Future studies are required to judge this hypothesis.

It is generally recognized that K^+ is essential for proper neuromuscular function and cellular metabolism. Insulin and catecholamines regulate the distribution of potassium between the extracellular and intracellular fluid compartments by activating the Na+-K+ ATPase [51]. Besides, insulin stimulates D-glucose intake into muscles and adipose tissue by activating the phosphatidylinositol 3-kinase pathway [36]**.** Hence, blood K^+ levels and glucose concentrations were addressed to determine the effect of the epidural insulin on these variables and allow in-depth understanding of the related findings.

In BUP group, blood glucose concentrations significantly increased from baseline (from 15-120 minutes). This could be explained by sedating the studied dogs with xylazine for epidural punctures [52]**.** Interaction of xylazine with postsynaptic α_2-adrenergic receptors of pancreatic β-cells with subsequent reduction of insulin release is the underlying mechanism [53-54]. In BUP/INS group, a bi-phasic alteration in blood glucose concentrations (significant increase followed by significant decrease at certain time points) was detected relative to baseline and even relative to BUP group. Initially elevated glucose concentrations relative to baseline could be attributed to xylazine administration altogether with dextrose infusion in this group which could also contribute to elevated concentrations compared to BUP group. To prevent the hypoglycemic effect of insulin, dextrose support was recommended in conjunction with intravenous insulin [55]. Accordingly, in the present study, dextrose was infused in BUP/INS group even though hypoglycemia was recorded at certain time points. In this study, hypoglycemia seemed not to be severe to negatively impact the overall results and the value of insulin as an antagonist despite optimizing dextrose supplement to avoid any hypoglycemia might upgrade the clinical significance of epidural insulin.

Blood K+ levels significantly increased from baseline at certain time points (30-120 minutes) in BUP group. Xylazine administered to the studied dogs might be implicated in such alteration whereas plasma concentrations of K^+ increased significantly following higher doses of xylazine in dogs [56]. Intravenous insulin reduces serum K^+ levels in a dose-dependent fashion [57]. Further, intravenous insulin combined with dextrose substantially lowered the serum potassium level for about 4-6 hours [85]. This was dependent on the theory that exogenous glucose stimulates insulin secretion which shifts potassium intracellularly [59]. Consequently, the reducing effect of insulin given in BUP/INS group might be balanced by the speculated xylazine associated increment in K^+ levels (demonstrated in BUP group) causing the insignificant changes in K^+ levels detected in BUP/INS group.

It has been shown that insulin affects vascular tissues both directly and indirectly. It mediates vasodilation by directly stimulating nitric oxide release from the endothelium [60]. It is well known that the cranial spread of the local anesthetic, which in turn depends on the anesthetic volume, concentration, injection velocity, amount of epidural fat, size of the epidural space, posture, and gravity, determines the onset and the duration of the sensory and motor block that results from a lumbosacral epidural injection [29–35]. With respect to local anesthetic volume, we hypothesized that if insulin given epidurally expressed its vasodilatory effect, it would enhance vascular uptake of epidural bupivacaine and secondly reduce its volume in epidural space and its duration of action. To effectively judge this point, serum concentrations of bupivacaine were compared among BUP and BUP/INS group.

Peak serum concentrations of bupivacaine achieved in BUP/INS and BUP groups were 0.29 Mg/ml and 0.28 Mg/ml, respectively. Higher concentrations (1.4 Mg/ml) were previously detected in dogs following epidural administration of bupivacaine [61]. In part, this difference could be attributed to lower doses used in the present study (1mg/kg) compared to the previous study (1.8 mg/kg) [61]. Difference in extent of vasculature in epidural space due to different study population might be another contributing factor.

In the present study, peak serum concentrations of bupivacaine were achieved faster in BUP/INS group. Serum concentrations were also significantly higher in BUP/INS group than BUP group at certain time points. These findings could denote that insulin enhanced blood uptake of epidural bupivacaine which necessarily participated in the shorter duration of sensory and motor blockade observed in this group compared to BUP group.

One of the limitations of this study was that despite animals were monitored for clinical neurological sequelae for considerable duration [27] especially as a single shot of insulin was tested, histological study is still insistent to rule out any microscopic evidence of neurotoxicity [27, 38].

Further, in the present study, the interval between the onset of action of bupivacaine (complete sensory and motor blockade) and epidural administration of insulin (to study its reversing effect) was 15 minutes which is different from the clinical scenario in which the proposed reversal technique would be applied at the end of anesthesia (to accelerate motor block recovery). Despites, this time schedule was chosen to characterize the reversal potential of insulin closer to its marked blocking effect, not to overestimate the overall results and also to obviate the need to assess similar reversal agent after various anesthesia durations experimentally which would expose tested animals to unnecessary stress. Lastly, in this study, insulin was administered via epidural route by second epidural puncture not systematically. Despite of the need to a second epidural puncture, this approach seems reasonable if applied clinically at end of anesthesia as no discomfort during injection would be encountered. This approach was further chosen as insulin concentration reaching targeted nerves was anticipated to exceed that would be achieved if insulin given systematically which is a major determinant for the overall reversal efficacy.

Conclusion

Epidural administration of insulin did not induce any clinical neurological sequelae in the studied dogs. The resultant analgesia (lumbar and sacral area dermatomes and toe web analgesia) and motor blockade following epidural bupivacaine were of shorter duration in BUP/INS group than BUP group. Hence, epidurally administered insulin was effective in reversing and enhancing the recovery from bupivacaine induced epidural anesthesia in dogs. Large scale studies are still required involving histological studies (to fully investigate the neurotoxicity of epidural insulin in dogs) and well-designed studies to evaluate (comprehensively evaluate sensory and motor block durations, cardiorespiratory parameters and biochemical indices) and validate the overall approach in clinical setting.

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Declaration of conflict of interest

The authors declare that there is no conflict of interest.

Ethical of approval

The protocol described in this manuscript was approved by the Institutional Animal Care and Use Committee of Faculty of Veterinary Medicine, University of Sadat City (no. VUSC-019-1-24). All methods and procedures followed the relevant guidelines and regulations of Institutional Animal Care and Use Committee of Faculty of Veterinary Medicine, University of Sadat City.

Author contributions

AH: Basic conceptualization of the project, data collection and sorting, interpretation of data and writing the primary version of the manuscript.
AM&DM: Data collection, interpretation of data and writing the primary version of the manuscript
SG & AM: Basic conceptualization of the project, interpretation of data and revise the manuscript
All authors approved the final manuscript.

TABLE 1. Simple descriptive scale (SDS) used for assessment of sedation in BUP (bupivacaine) and BUP/INS (bupivacaine/insulin) groups

SDS	Degree of sedation
0	No sedative effect: Fully responsive, no signs of depression or drowsiness
1	Mild sedation: Responsive with slow or reduced reaction to stimuli (some response to acoustic stimuli), mild signs of depression and drowsiness
2	Moderate sedation: Markedly slow or minimal reaction to stimuli (minimal response to acoustic stimuli), moderate signs of depression and drowsiness
3	Marked sedation: Lateral or sternal recumbency with inability to stand on the four limbs, unresponsive to stimuli (no response to acoustic stimuli), very depressed, very drowsy and sleepy

TABLE 2. Simple descriptive scale (SDS) used for assessment of analgesia in left hind toe web and lumbar and sacral areas in BUP (bupivacaine) and BUP/INS (bupivacaine/insulin) groups

Region	SDS	Degree of analgesia
Left hind toe web	0	No analgesia: Normal response: Pelvic limb withdrawal and/or vocalization
	1	Partial analgesia: Reduced response
	2	Complete analgesia: No response
Lumbar and sacral areas	0	No analgesia: Normal response: Skin contraction
	1	Complete analgesia: No response

TABLE 3. Simple descriptive scale (SDS) used for assessment of motor blockade in BUP (bupivacaine) and BUP/INS (bupivacaine/insulin) groups

SDS	Degree of motor blockade
0	No motor blockade: Normal motor response: Normal ability to walk or stand using the hind limb
1	Partial motor blockade: Movement in hind limb, some weight bearing, presence of ataxia
2	Complete motor blockade: Paralysis of hind limb

Table 4. Median (range) of sedation and analgesia scores in BUP (bupivacaine) and BUP/INS (bupivacaine/insulin) groups

Time	Groups	Variables			
		Sedation scores	**Toe web analgesia scores**	**Sacral area analgesia scores**	**Lumbar area analgesia scores**
Baseline	BUP	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	BUP/INS	0(0-0)	0(0-0)	0(0-0)	0(0-0)
5min	BUP	2(2-3)*	1(1-1)*	1(1-1)*	1(1-1)*
	BUP/INS	3(2-3)*	1(1-1)*	1(1-1)*	1(1-1)*
15min	BUP	2(1-3)*	2(2-2)*	1(1-1)*	1(1-1)*
	BUP/INS	2(2-3)*	2(2-2)*	1(1-1)*	1(1-1)*
30min	BUP	2(1-2)*	2(2-2)*	1(1-1)*	1(0-1)*
	BUP/INS	2(1-2)*	2(2-2)*	1(1-1)*	0(0-1)
45min	BUP	1(1-2)*	2(2-2)*	1(1-1)*	1(0-1)
	BUP/INS	1(1-2)*	2(2-2)*	1(0-1)*	0(0-1)
60min	BUP	1(1-2)*	2(2-2)*	1(0-1)*	0(0-1)
	BUP/INS	1(0-1)	2(1-2)*	1(0-1)*	0(0-1)
75min	BUP	0(0-2)	2(1-2)*	1(0-1)*	0(0-0)
	BUP/INS	1(0-1)	2(1-2)*	1(0-1)	0(0-0)
90min	BUP	0(0-2)	2(1-2)*	1(0-1)*	0(0-0)
	BUP/INS	1(0-1)	1(1-2)*	1(0-1)	0(0-0)
105min	BUP	0(0-1)	1(1-2)*	1(0-1)	0(0-0)
	BUP/INS	0(0-0)	1(1-2)*	0(0-1)	0(0-0)
120min	BUP	0(0-1)	1(1-2)*	0(0-1)	0(0-0)
	BUP/INS	0(0-0)	1(0-2)*	0(0-1)	0(0-0)
150min	BUP	0(0-0)	1(1-2)*	0(0-1)	0(0-0)
	BUP/INS	0(0-0)	1(0-2)	0(0-0)	0(0-0)
180min	BUP	0(0-0)	1(0-2)	0(0-0)	0(0-0)
	BUP/INS	0(0-0)	0(0-2)	0(0-0)	0(0-0)
210min	BUP	0(0-0)	1(0-2)	0(0-0)	0(0-0)
	BUP/INS	0(0-0)	0(0-2)	0(0-0)	0(0-0)
240min	BUP	0(0-0)	1(0-2)	0(0-0)	0(0-0)
	BUP/INS	0(0-0)	0(0-1)	0(0-0)	0(0-0)
270min	BUP	0(0-0)	0(0-1)	0(0-0)	0(0-0)
	BUP/INS	0(0-0)	0(0-1)	0(0-0)	0(0-0)
300min	BUP	0(0-0)	0(0-1)	0(0-0)	0(0-0)
	BUP/INS	0(0-0)	0(0-1)	0(0-0)	0(0-0)
330min	BUP	0(0-0)	0(0-1)	0(0-0)	0(0-0)
	BUP/INS	0(0-0)	0(0-1)	0(0-0)	0(0-0)
360-720min	BUP	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	BUP/INS	0(0-0)	0(0-0)	0(0-0)	0(0-0)

*Significantly different ($p < 0.05$) from the corresponding baseline. Results of the shown variables from 360-720 minutes were compiled as from 360-time point, there were no changes relative to baseline in any of these variables.

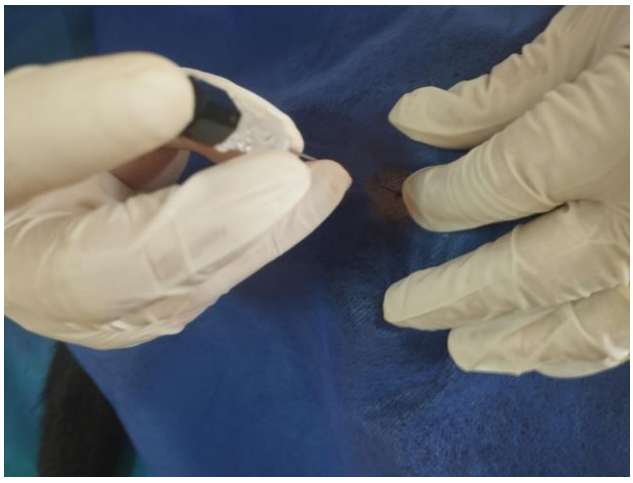

Fig. 1. Puncture in the lumbosacral space in a dog using a 22-gauge, 90mm spinal needle.

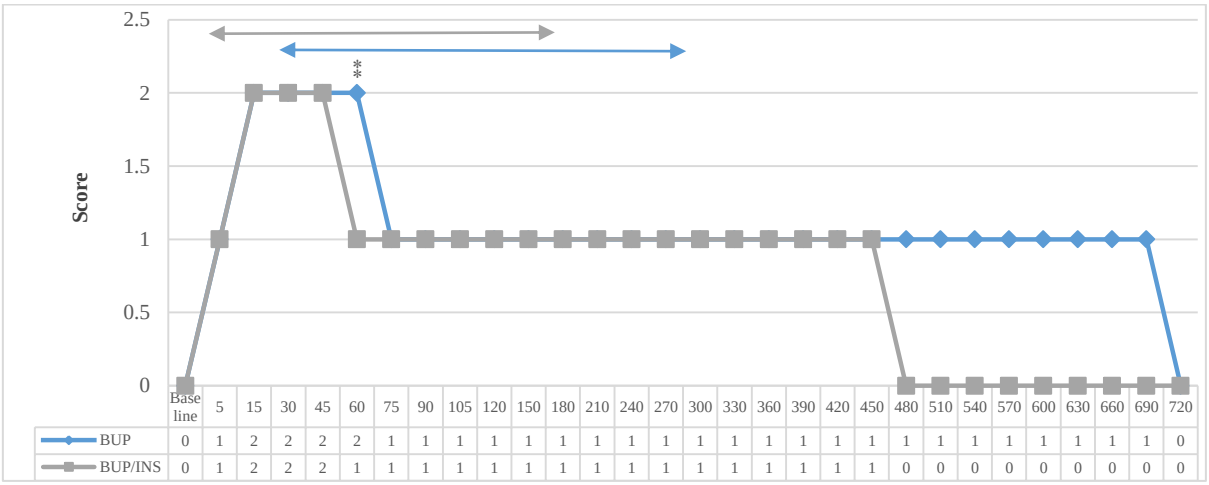

Fig. 2. Median of motor blockade scores in BUP (bupivacaine) and BUP/INS (bupivacaine/insulin) groups.

Significant difference from baseline in BUP group.

Significant difference from baseline in BUP/INS group.

*Significant difference between BUP and BUP/INS at this time point.

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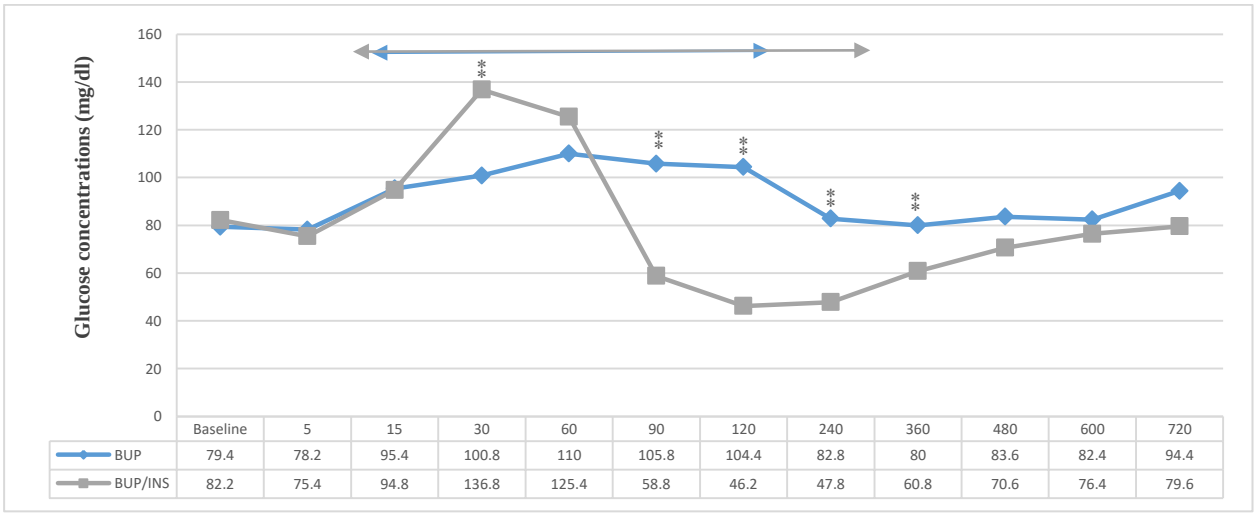
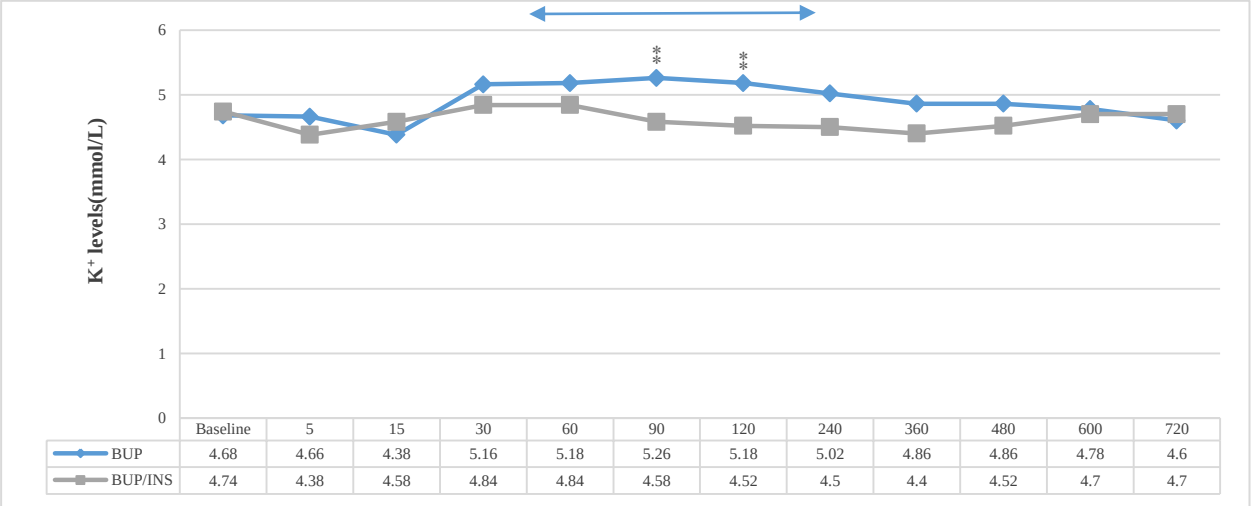


Fig. 3. Mean of blood glucose concentrations in BUP (bupivacaine) and BUP/INS (bupivacaine/insulin) groups.

Significant difference from baseline in BUP group. Significant difference from baseline in BUP/INS group.

*Significant difference between BUP and BUP/INS.

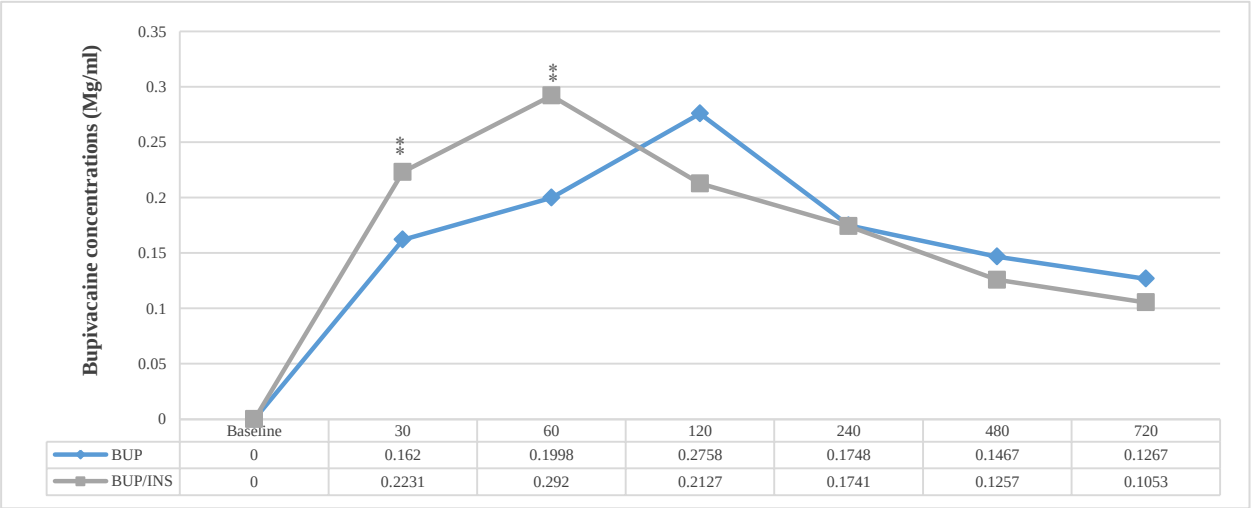


	Baseline	5	15	30	60	90	120	240	360	480	600	720
BUP	4.68	4.66	4.38	5.16	5.18	5.26	5.18	5.02	4.86	4.86	4.78	4.6
BUP/INS	4.74	4.38	4.58	4.84	4.84	4.58	4.52	4.5	4.4	4.52	4.7	4.7

Fig. 4. Mean of blood K^+ (potassium) levels in BUP (bupivacaine) and BUP/INS (bupivacaine/insulin) groups.

↔ Significant difference from baseline in BUP group.

↔ ** Significant difference between BUP and BUP/INS.



	Baseline	30	60	120	240	480	720
BUP	0	0.162	0.1998	0.2758	0.1748	0.1467	0.1267
BUP/INS	0	0.2231	0.292	0.2127	0.1741	0.1257	0.1053

Fig. 5. Mean serum concentrations of bupivacaine in BUP (bupivacaine) and BUP/INS (bupivacaine/insulin) groups. Significant difference from baseline in BUP group.

↔ Significant difference from baseline in BUP/INS group.

↔ * Significant difference between BUP and BUP/INS.

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التأثير العكسي للأنسولين على التأثير المسكن والمانع للحركة للبيوبيفاكايين فوق الجافية في الكلاب

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الملخص

يعد التخدير فوق الجافية تقنية شائعة في الطب البيطري وذلك على الرغم من عدم وجود عامل عكسي محدد لمواد التخدير الموضعي وهوما يشكل مجموعة من التحديات. لذلك قامت هذه الدراسة بتقييم الأنسولين كعامل عكسي للبيوبيفاكيين فوق الجافية في الكلاب. وقد اشتملت الدراسة علي مرحلتين (المرحلة 1 و 2). المرحلة 1: أجريت على ثلاثة كلاب لتحديد التأثير العصبي للأنسولين المعطى عن طريق فوق الجافية ولتوضيح جرعة الأنسولين العاكسة الفعالة. المرحلة 2: شملت ستة كلاب تلقوا علاجين عن طريق فوق الجافية بفاصل أسبوع واحد. وقد تم تقييم مجموعتين في تلك المرحلة: مجموعة البيوبيفاكيين (بيوبيفاكيين فوق الجافية 1 ملغم / كغم + محلول ملحي وتسريب ملحي) و مجموعة البيوبيفاكيين/ أنسولين (بيوبيفاكيين فوق الجافية 1 ملغم / كغم + الأنسولين 1 وحدة دولية / كغم وتسريب دكستروز). وقد تم تقييم كلتا المجموعتين من حيث التهدئة والتسكين ومنع الحركة وتركيزات الجلوكوز والبوتاسيوم في البلازما وكذلك تركيزات البيوبيفاكيين في المصل. لم تكشف المرحلة الأولى عن أي مضاعفات عصبية إكلينيكية بعد حقن الأنسولين فوق الجافية واقترحت جرعة 1 وحدة دولية/كغم من الأنسولين كجرعة عكسية. وقد كشفت المرحلة الثانية عن مدة ودرجة مماثلة من التهدئة في كلتا المجموعتين بينما كانت مدة التسكين ومنع الحركة مطولة في مجموعة البيوبيفاكيين مقارنة بمجموعة البيوبيفاكيين/ أنسولين. وقد تم الكشف عن تغيرات كبيرة في تركيزات الجلوكوز في الدم في كلتا المجموعتين مقارنة بالتركيزات التي سبقت اعطاء اي من الادوية اضافة الي وجود اختلافات كبيرة في تركيزات الجلوكوز بين المجموعتين. وقد حدث تغيرفي تركيزات البوتاسيوم بشكل كبير فقط في مجموعة البيوبيفاكيين مقارنة بالتركيزات قبل اعطاء الادوية .وقد خلصت الدراسة الي ان الأنسولين المعطي عن طريق فوق الجافية قد عكس بشكل فعال تأثير المنع الحسي والحركي للبيوبيفاكيين فوق الجافية في الكلاب.

الكلمات الدالة: مسكنات الألم، الكلاب، بيوبيفاكيين فوق الجافية، الأنسولين، تأثير منع الحركة، التأثير العكسي.