

## Original Article

# Effects of clonidine and intrathecal dexmedetomidine under ropivacaine spinal anesthesia

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**Abstract:** Analgesia with local anesthetics has been proved to be effective in neuraxial block. However neural complications and toxicity associated with local anesthetics lead to the use of adjuvants that reduce dose levels and minimise toxicity of anesthetics. This study aims to assess the effects of intrathecal dexmedetomidine and clonidine under ropivacaine anesthesia. P7 and P21 Sprague-Dawley rat pups were exposed to spinal anesthesia of 0.5% ropivacaine. Separate groups of rats were given intrathecal clonidine (0.03 or 0.1 mg/kg b.wt) or dexmedetomidine (3 or 10 µg/kg b.wt) dose along with ropivacaine. Immunohistochemistry for activated-caspase-3 expression and TUNEL assay were performed to assess neuroapoptosis in the spinal sections. Glial reactivity was measured by detecting glial fibrillary acidic protein and ionized calcium binding adapter molecule 1. The animals were tested for sensory and motor blocks following anesthesia. Intrathecal injections of ropivacaine in combination with clonidine or dexmedetomidine resulted in dense blocks and as well extended the duration of spinal blocks. The latency of fall was shorter in rats exposed to ropivacaine when compared with clonidine or dexmedetomidine administration with ropivacaine spinal anesthesia. The apoptotic cell counts and glial activity was markedly lower in rats that received combined doses of clonidine or dexmedetomidine compared to the single drug injection of ropivacaine. Clonidine or dexmedetomidine administration with ropivacaine increased the intensity and duration of spinal blockade thereby increased anesthetic effects and lowered ropivacaine induced neurotoxicity.

**Keywords:** Anesthesia, clonidine, dexmedetomidine, ropivacaine, spinal toxicity

## Introduction

Spinal anesthesia is commonly employed in surgical practice. Ropivacaine is often chosen over other drugs as mepivacaine or lidocaine in an effort to extend the duration of postoperative analgesia and to prolong surgical block as well [1]. Nevertheless, the advantages of longer-duration blocks are numerous, particularly in the postoperative setting. The associated possibility of both local and systemic toxicity rules out a small increase in the drug concentration and/or volume of administration as a means to extend analgesic duration [1]. In an effort to broaden the narrow therapeutic window of local anesthetics and to increase the duration of analgesia, several adjuvants have been studied for a given local anesthetic towards reducing the dose and/or minimizing other effects like neurotoxicity. These adju-

vants include clonidine, buprenorphine, dexamethasone and midazolam [2-4].

α<sub>2</sub>-adrenergic agonists have both analgesic and sedative properties when used as an adjuvant in regional anesthesia [5, 6]. These drugs potentiate the effect of local anesthetics and also prolong the duration of both motor, sensory spinal blockade and postoperative analgesia [7-9].

Clonidine, a partial α<sub>2</sub>-adrenoreceptor agonist is used intrathecally, with a well-established record of efficacy and safety [7]. Dexmedetomidine is a highly selective α<sub>2</sub>-adrenergic agonist with better hypnotic, sedative, and analgesic activities. It has been used safely for general anesthesia and postoperative analgesia without any respiratory depression [10]. Dexmedetomidine was found to possess α<sub>2</sub>/α<sub>1</sub> selectiv-

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ity ratio of 1600:1 and is more potent than clonidine [11]. Intrathecal dexmedetomidine has been reported to produce analgesia in experimental animals [12], prolongation in duration of spinal bupivacaine [13] and also found to potentiate the effect of spinal morphine during cancer pain in humans [14]. The present study represents an effort to study the effects of clonidine and dexmedetomidine on ropivacaine anesthesia in neonatal rats.

### Materials and methods

#### *Chemicals and reagents*

All the chemicals and reagents used in the study were of analytical grade and were obtained from Sigma-Aldrich, St. Louis, MO, USA, unless otherwise mentioned.

#### *Animals*

The study was approved by the Institutional Animal Care Committee and performed in accordance with the National Institutes of Health Guide for the Use of Laboratory Animals. Pregnant Sprague Dawley rats (Guangdong Medical Laboratory Animal Co., China) used in this study was maintained on a 12 h light-dark cycle (lights on from 07:00 to 19:00) at room temperature at  $(21 \pm 1^\circ\text{C})$ . The rats were provided *ad libitum* access to water and food. The animals were housed individually in separate cages and monitored closely for the day of birth, which was considered as postnatal day 0 (P0). The pups were kept in cages in a room on a 12 h light/dark cycle with free access to water with their littermates. Postnatal (P) day 7 and P21 female rat pups were assigned separate treatment groups (n=12).

#### *Injections of ropivacaine, clonidine and dexmedetomidine*

Pups were anesthetized with isoflurane (3-5%) in oxygen and air. Percutaneous intrathecal injections were made at the low lumbar level (intervertebral space L4-5 or L5-L6) with a 30-gauge needle perpendicular to the skin. Injective volumes of 0.5  $\mu\text{L}$  per gram bodyweight (previously determined to produce spread across lumbar and low thoracic segments in rat pups [15]), were delivered using a 50  $\mu\text{L}$  Hamilton syringe. Ropivacaine at 0.5% [16, 17] was administered to the rats. Control rats received no anesthesia. The treatment pups re-

ceived ropivacaine or ropivacaine along with clonidine at 0.03 or 0.1 mg/kg [18] or dexmedetomidine at 3 or 10  $\mu\text{g}/\text{kg}$  [19].

#### *Evaluation of neuroapoptosis*

Apoptosis following exposure to anesthesia was evaluated by TUNEL assay and by immunohistochemical detection for activated caspase-3. Rats were transcardially perfused with 0.1 M phosphate buffer containing 4% paraformaldehyde, 6 h after anesthesia. After gentle dissection of the spinal cord, the tissues were stored overnight at  $4^\circ\text{C}$  in 4% paraformaldehyde and then transferred to a 30% sucrose solution at  $4^\circ\text{C}$  until sectioning. The transverse sections of lumbosacral spinal cord (7 and 14  $\mu\text{m}$ ) were excised using a cryostat and fixed on slides and stored at  $-30^\circ\text{C}$ .

Immunohistochemistry was performed as described previously [20] with minor modifications. The slides were incubated for about 10 min in 3% peroxidase and blocked with 0.3% Triton X-100 and 5% normal goat serum in Tris-buffered saline at room temperature for 60 min, followed by incubation with rabbit monoclonal anti-activated caspase 3 (1:100; Cell Signaling, Danvers, MA, USA) overnight at  $4^\circ\text{C}$ . After incubation with primary antibody, biotinylated goat anti-rabbit secondary antibody (1:200, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was applied and incubated further for 40 min at room temperature. The slides were further incubated with avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA) for 40 min. Tissue sections were stained with diaminobenzidine for about 8 min. The slides were counterstained with hematoxylin, dehydrated and observed. Cleaved caspase-3 positive cells were analyzed using NIS-Elements BR imaging processing and analysis software (Nikon Corporation, Japan).

TUNEL assay was performed using the Dead End TM fluorometric TUNEL system kit (Promega, Madison, WI, USA) as described [21]. Briefly, the slides were protected from direct light during experiment and Hoechst stain was used. TUNEL positive cells (stained nuclei) were analyzed as mentioned in the immunohistochemistry staining. TUNEL positive cell counts were calculated by dividing the number of TUNEL positive cells by the area of the observed region.

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### *Glial fibrillary acidic protein and ionized calcium binding adapter molecule 1*

Spinal cords sections (14  $\mu\text{m}$  thick) 7 days following anesthesia injections were evaluated for glial reactivity with astrocyte (glial fibrillary acidic protein) and microglial (Iba1) markers. The slides were washed with Triton X-100 (0.1%) in phosphate buffered saline, and incubated in 5% goat blocking serum at room temperature for 60 min. Following incubation, the slides were treated with mouse anti-glial fibrillary acidic protein (1:500; Chemicon, Temecula, CA, USA) and rabbit anti-Iba1 (1:1000; WAKO, Richmond, VA, USA) for 48 h at 4°C and further with fluorescent secondary antibodies for 2 h (1:250 goat anti-mouse Alexa 555 and 1:250 goat anti-rabbit Alexa 488; Molecular Probes, Eugene, OR, USA). Slides were then treated with 4',6-diamidino-2-phenylindole (DAPI). The fluoresce were detected in at least 3 sections from each animal using fluorescence illuminator (Olympus America Inc., Center Valley, PA, USA) equipped with a digital camera and image-capture software. The mean intensity of immunofluorescence within a fixed region of interest in the dorsal horn and background intensity was calculated using Image Pro Plus software (Media Cybernetics Inc., Silver Spring, MD, USA) [18].

### *Response to mechanical and thermal stimulus*

The P7 and P21 rats underwent baseline measurement of hind paw thermal withdrawal latencies immediately before spinal injection. Sensory blockade was assessed for thermal hyperalgesia using a modified hot plate test as described previously [22, 23]. Hind paws of the rat pups were exposed in sequence (left then right) to a hot plate (model 39D hot plate analgesia meter; IITC Inc., Woodland Hills, CA, USA) at 51°C for P7 and 55°C for P21 pups. The time from the initiation of stimulus (thermal withdrawal latency) until the rats lifted their paws was measured with a stopwatch. After 12 sec, the tested paw was removed to avoid any tissue damage to the animal due to development of hyperalgesia. The test was repeated three times (with a 10 sec pause between tests) for each rat at every time point. Thermal withdrawal latencies were measured every 10 min for at least 40 min after the intrathecal injection. Thermal withdrawal latency was taken as the average of 3 measures from each hind paw.

Response of the rat pups following mechanical stimulus was also assessed. Mechanical blockade was recorded using hind paw withdrawal with von Frey filaments. The P7 and P21 rats were lightly restrained on a flat surface and were delivered mechanical stimuli. von Frey hairs device (electronic von Frey device, Stoelting, Wood Dale, IL, USA) that deliver increasing mechanical stimuli was applied to the dorsal surface of the hind paw of the pups, five times with one second intervals [24]. The number of evoked withdrawal responses to each stimulus of increasing intensity was recorded until a given stimulus evoked five responses, or until a supra threshold cut-off pressure was reached [24]. Mechanical withdrawal thresholds were recorded at baseline and every 10 min for at least 60 min after the intrathecal injection.

Motor performance of the lower extremities was further assessed by a qualitative pinch score. For each limb, if there was no spontaneous or evoked movement, the contribution to the score was zero. If any partial movement was observed, the contribution to the score was noted as one, and if there was normal movement, the contribution to the score was two. Thus, in summing the values, the score could range from zero (complete blockade) to four (normal).

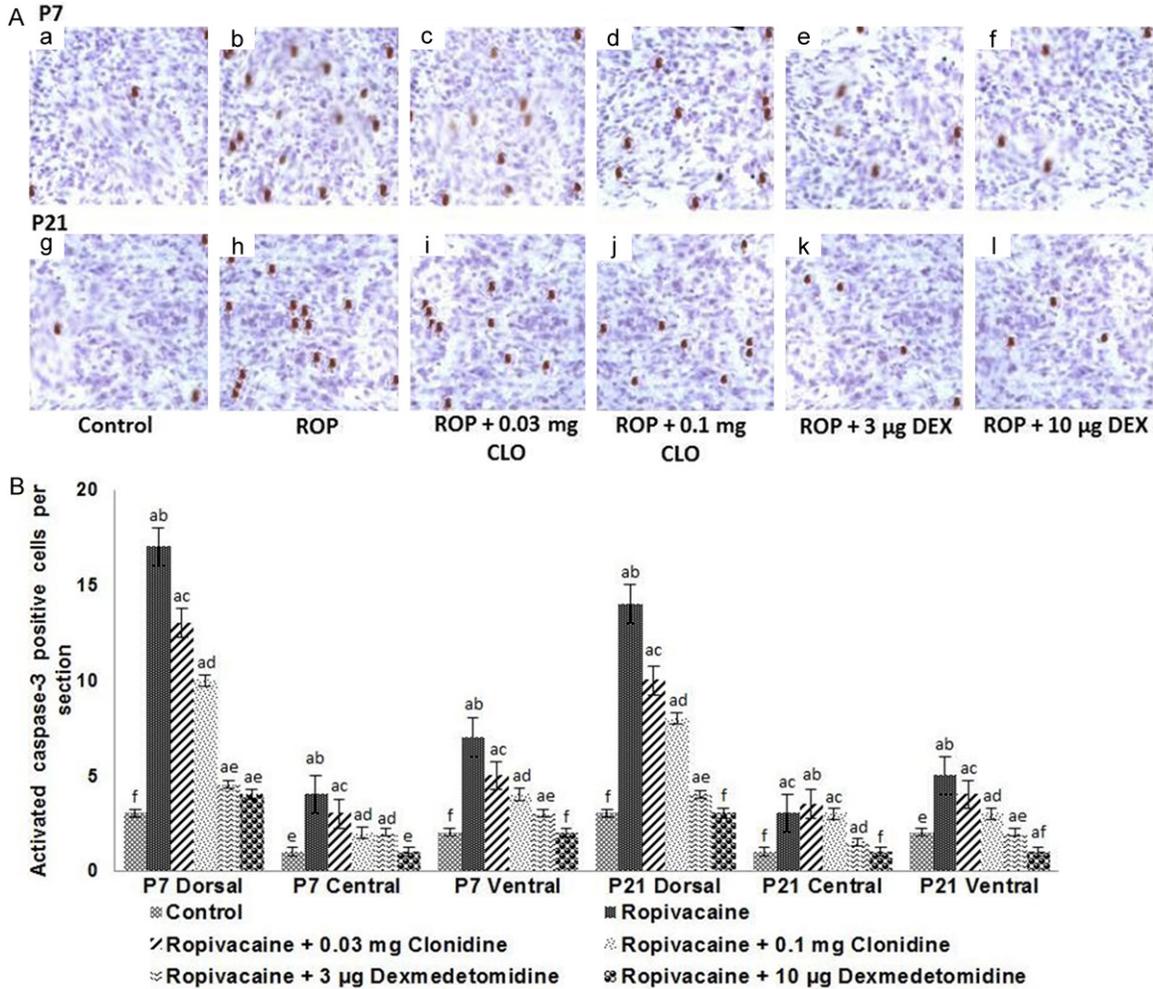
### *Motor behaviour*

Motor impairment of the rats was assessed 12 h. The rats that had undergone spinal injections on P7 or P21 were assessed for the motor behaviour. The rats were placed on a dual species Economex Rotarod (Columbus Instruments, Columbus, OH) and subjected to rotating motion using a spindle rotating at 10 rotations per minute [25]. Each rat was tested thrice for its capacity to rotate and stay on the spindle. A time interval of 10 min was used between each assessment. The maximal latency for each trial was 300 sec before removal from the spindle and the latency of fall was recorded. Average of the three assessments was used for data analysis.

### *Statistical analysis*

All the values are represented as mean  $\pm$  SD. Values at  $P < 0.05$  are considered significant as determined by one-way Analysis of Variance

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**Figure 1.** Activated caspase-3 positive cells in the spinal cord sections of rat pups following intrathecal anesthesia. (A) Dorsal sections of the spinal tissue presenting activated caspase-3 positive cells. Activated caspase-3 positive cell counts in P7 (a-f) and P21 (g-l) rats following anesthesia. (ROP-Ropivacaine; CLO-Clonidine; DEX-Dexmedetomidine) (B) Caspase-3 positive cell count Data are given as mean  $\pm$  SD where  $n = 3$ . <sup>a</sup>denotes statistical significance at  $P < 0.05$  related against control and <sup>b-f</sup>denotes data within the same group that differ from each other at  $P < 0.05$  as calculated by one-way ANOVA followed by DMRT analysis.

(ANOVA) followed by posthoc analysis by Duncan's Multiple Range Test (DMRT). The values were analyzed using SPSS software, version 17.0.

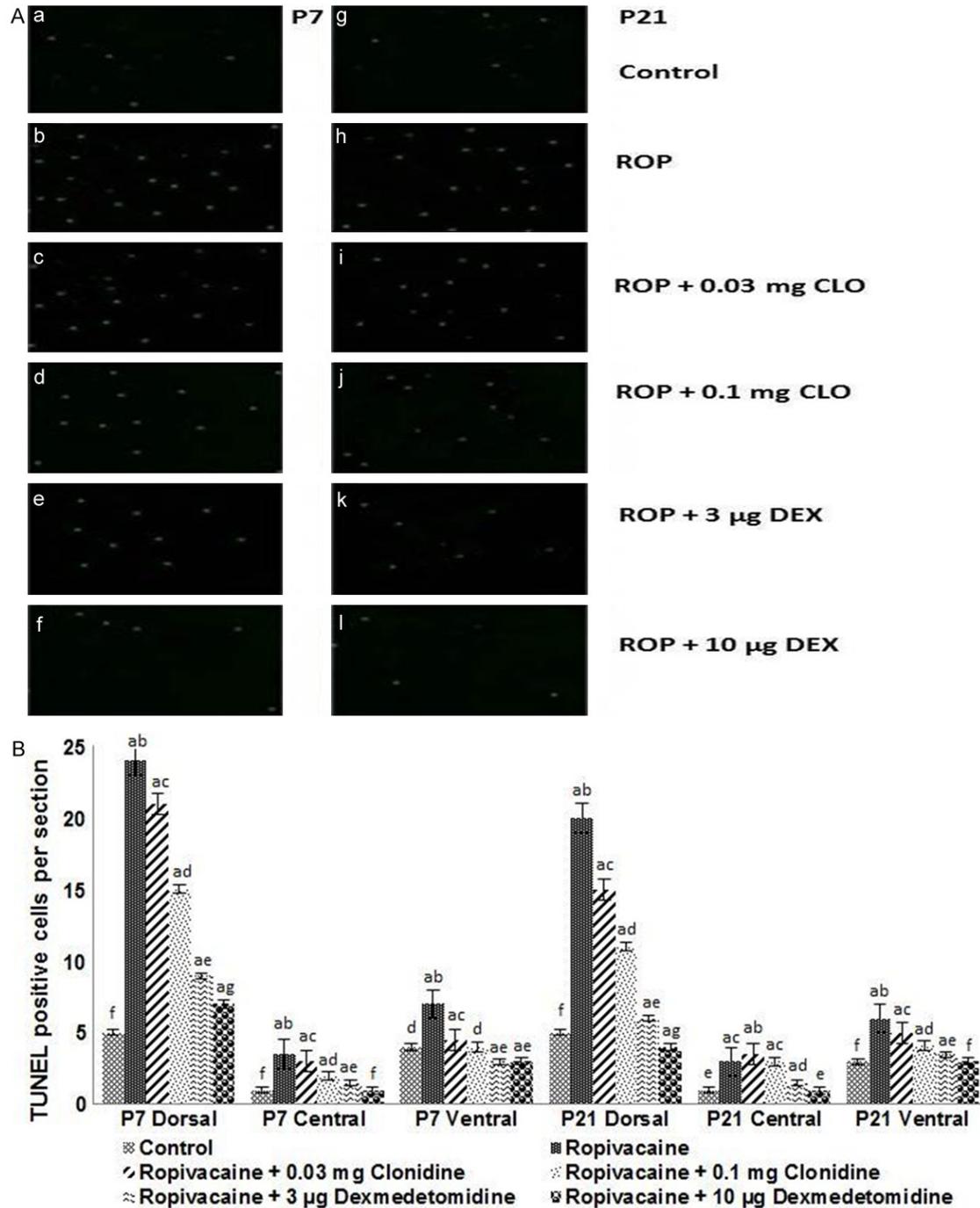
### Results

#### *Intrathecal anesthetics on neuroapoptosis*

Immunohistochemistry with antibody to activated caspase-3 was performed to study the expression of cleaved caspase-3, the final member of an intracellular cascade activated during programmed cell death. Cleaved caspase-3 expression is used as a marker of neuroapoptosis and cell death [21, 26, 27].

In our study, the intrathecal injections of ropivacaine exhibited significantly higher levels of caspase-3 positive cells (**Figure 1**). However, on combination doses with clonidine or dexmedetomidine, the counts were comparatively lower. While clonidine at both the doses resulted in a slight increase in caspase-3 positive cells than dexmedetomidine, combination doses of ropivacaine with dexmedetomidine resulted in a negligible raise in positive cell counts as compared with control rat pups that received no anesthesia. Further the caspase-3 positive cell counts were observed to be markedly higher in P7 rat pups that were exposed to intrathecal anesthetics irrespective of whether

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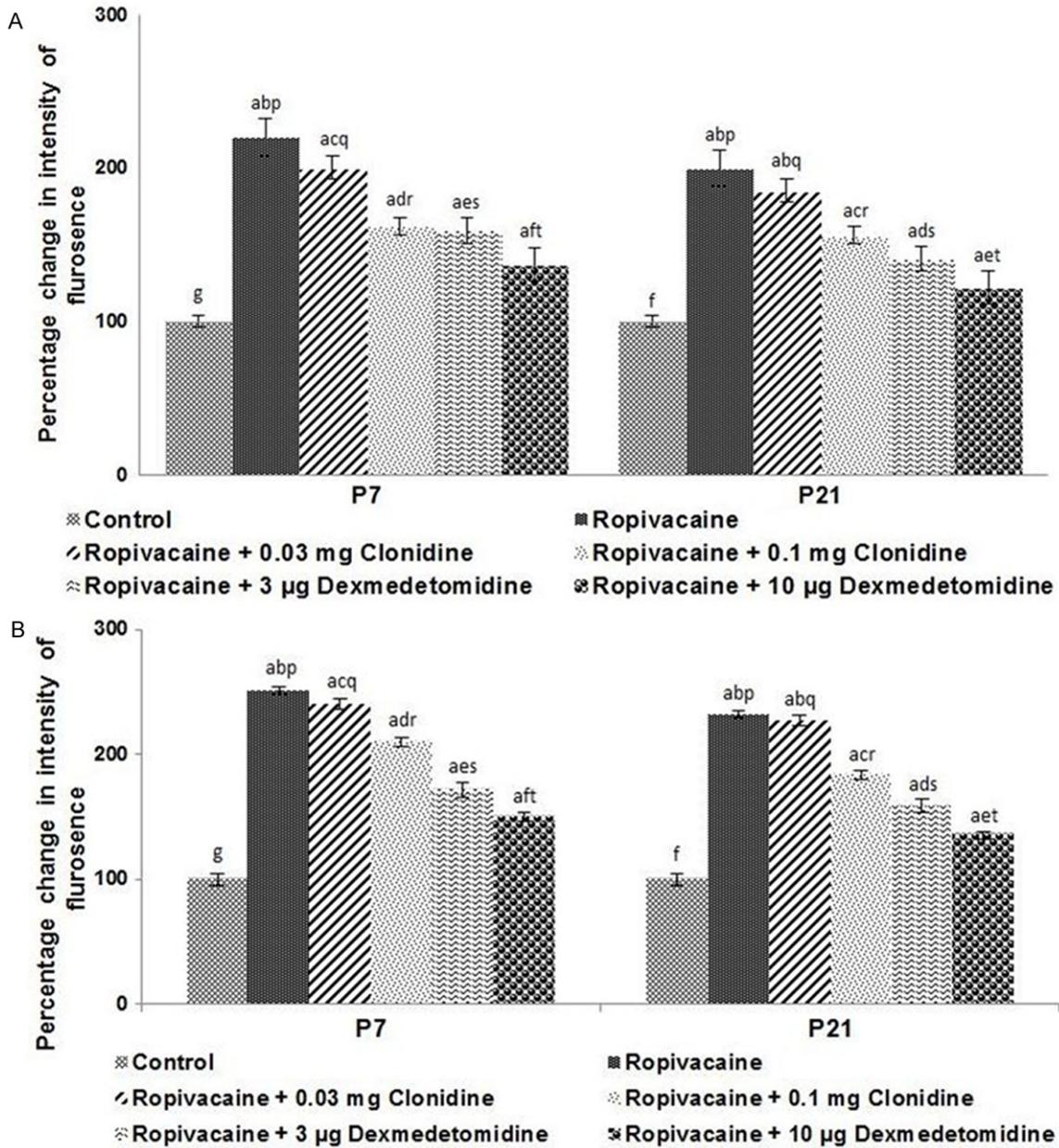


**Figure 2.** TUNEL positive cells in the spinal cord sections of rat pups following intrathecal anesthesia. (A) Dorsal sections of the spinal tissue presenting activated TUNEL positive cells in P7 (a-f) and P21 (g-l) rats following anesthesia. (a and g- Control; b and h- Ropivacaine; c and i- Ropivacaine+0.03 mg Clonidine; d and j- Ropivacaine+0.1 mg Clonidine; e and k- Ropivacaine+3 µg Dexmedetomidine; f and l- Ropivacaine+10 µg Dexmedetomidine). (B) Apoptotic cell counts. Data are given as mean ± SD where n = 3. <sup>a</sup>denotes statistical significance at P<0.05 related against control and <sup>b-e</sup>denotes data within the same group that differ from each other and <sup>p1</sup>refers to statistical difference between the same treatment groups of P7 and P21 animals as calculated by one-way ANOVA followed by DMRT analysis.

they received ropivacaine alone or ropivacaine along with clonidine or dexmedetomidine at both the doses.

We observed a marked raise in the apoptotic neuron counts in the spinal tissues of the rat pups exposed to anesthesia (**Figure 2**).

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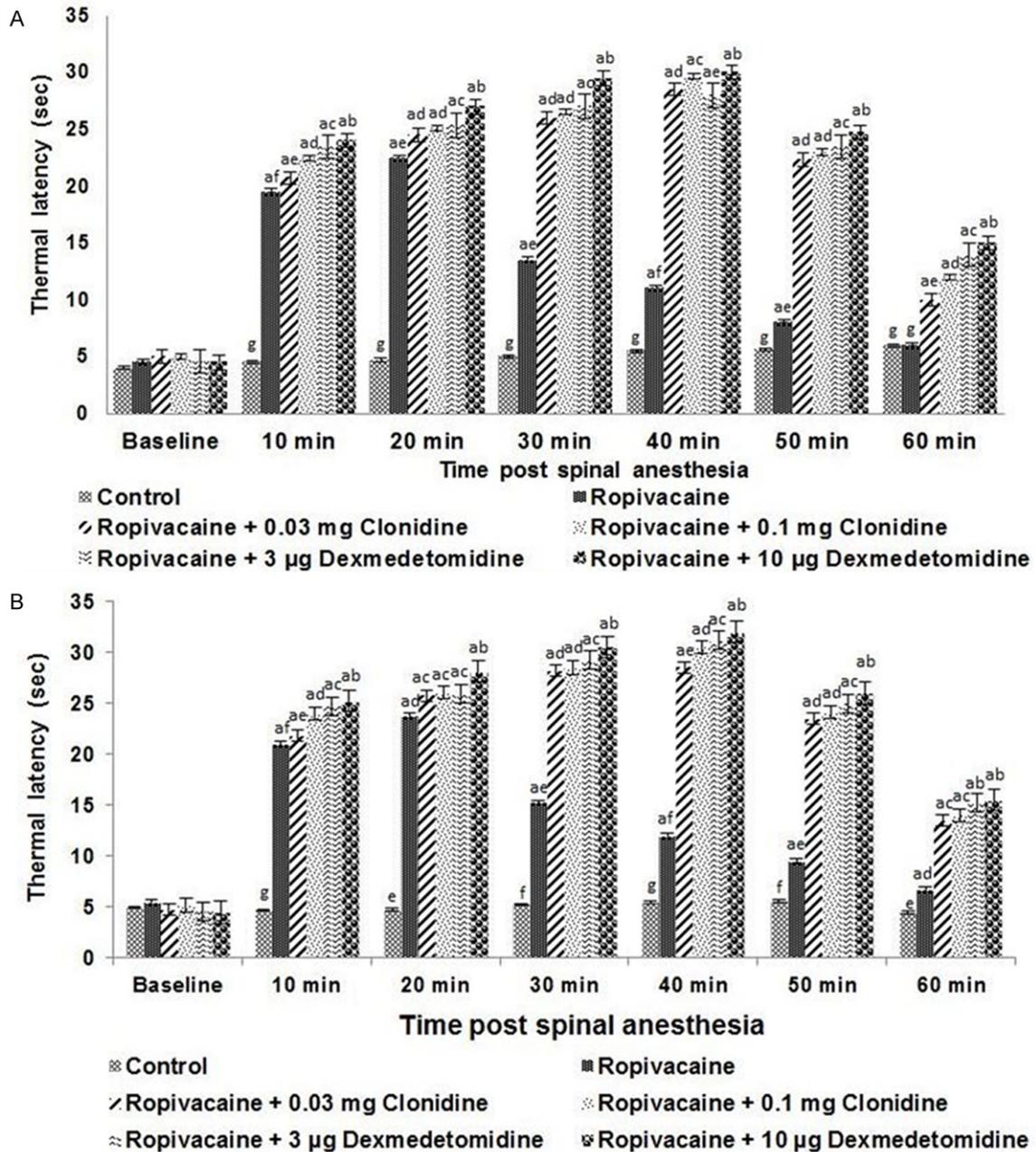


**Figure 3.** Glial reactivity levels in P7 and P21 rat pups following spinal anesthesia. Data are given as mean  $\pm$  SD where  $n = 3$ . <sup>a</sup>denotes statistical significance at  $P < 0.05$  related against control and <sup>b</sup>denotes data within the same group that differ from each other and <sup>p</sup>refers to statistical difference between the same treatment groups of P7 and P21 animals as calculated by one-way ANOVA followed by DMRT analysis.

Dexmedetomidine or clonidine combinations with ropivacaine resulted in a considerable decrease in the TUNEL positive cells. Higher doses of both dexmedetomidine and clonidine were able to cause a significant reduction in the apoptotic cell counts comparing with the lower concentrations respectively. Furthermore, the TUNEL positive cell counts were observed

to be the highest in P7 rat pups exposed to ropivacaine when it was administered alone. In the P21 rat pups, ropivacaine induced apoptosis was considerably lesser than in P7. Combined doses of dexmedetomidine and ropivacaine was much effective than combination with clonidine in both P7 and P21 rats. Immunohistochemistry and TUNEL assay studies showed

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**Figure 4.** Thermal latency of (A) P7 and (B) P21 rat pups following spinal anesthesia. Data are given as mean  $\pm$  SD where  $n = 3$ . <sup>a</sup>denotes statistical significance at  $P < 0.05$  related against control and <sup>b-f</sup>denotes data within the same group that differ from each other at  $P < 0.05$  as calculated by one-way ANOVA followed by DMRT analysis.

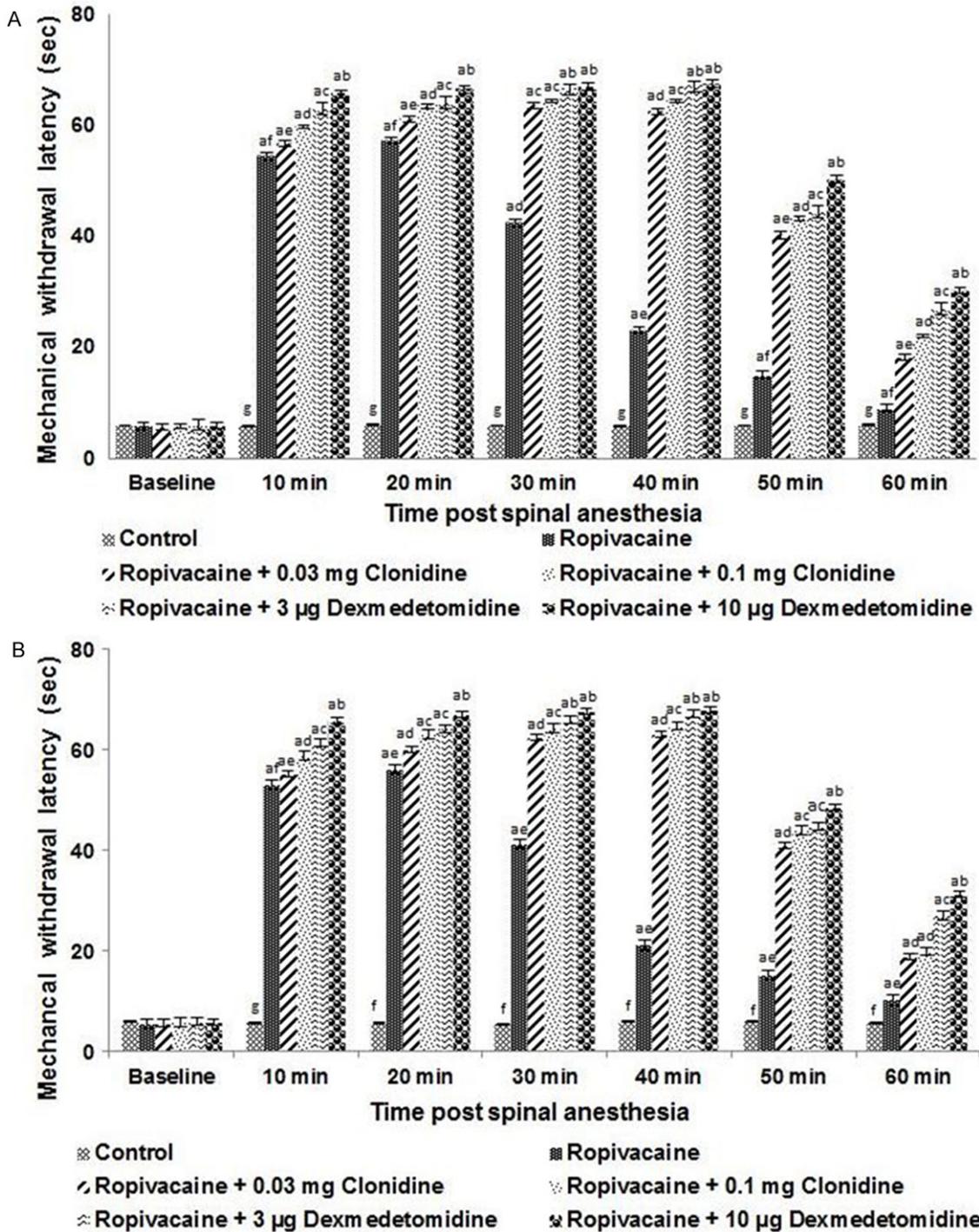
dexmedetomidine at higher dose in combination with ropivacaine exhibiting negligible neurotoxic effects than clonidine.

Consistent with the results of activated caspase-3 and TUNEL assay, the glial reactivity raised in the P7 rats exposed to anesthesia as against control rats. However, not much alteration was observed in P21 rats (Figure 3).

Dexmedetomidine or clonidine administered along with ropivacaine caused a decrease in the glial reactivity levels.

### Response to thermal and mechanical stimulus

Hind paw thermal withdrawal latencies were determined for P7 and P21 rat pups following intrathecal injections and the results are shown



**Figure 5.** Mechanical withdrawal threshold of (A) P7 and (B) P21 rat pups following spinal anesthesia. Data are given as mean  $\pm$  SD where  $n = 3$ . <sup>a</sup>denotes statistical significance at  $P < 0.05$  related against control and <sup>b-f</sup>denotes data within the same group that differ from each other at  $P < 0.05$  as calculated by one-way ANOVA followed by DMRT analysis.

in **Figure 4**. Ropivacaine either given alone or in combinations with clonidine or dexmedetomidine produced dense thermal nociceptive

blockade at 10 min following injection compared to control, and remained dense till 20 min. However, combined administrations were

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**Table 1.** Motor Block score after spinal anesthesia

Motor blocks in P7 rat pups following intrathecal injections							
	Time post anesthesia (min)						
	0	5	10	20	30	40	50
Control	4	4	4	4	4	4	4
Ropivacaine	0	0	0	2	2	4	4
Ropivacaine + 0.03 mg Clonidine	0	0	0	1	1	2	4
Ropivacaine + 0.1 mg Clonidine	0	0	0	1	1	2	4
Ropivacaine + 3 µg Dexmedetomidine	0	0	0	0	1	1	2
Ropivacaine + 10 µg Dexmedetomidine	0	0	0	0	0	1	2

Motor blocks in P21 rats pups following intrathecal injections							
	Time post anesthesia (min)						
	0	5	10	20	30	40	50
Control	4	4	4	4	4	4	4
Ropivacaine	0	0	0	2	2	4	4
Ropivacaine + 0.03 mg Clonidine	0	0	0	1	2	2	4
Ropivacaine + 0.1 mg Clonidine	0	0	0	1	2	2	4
Ropivacaine + 3 µg Dexmedetomidine	0	0	0	0	1	2	4
Ropivacaine + 10 µg Dexmedetomidine	0	0	0	0	1	2	2

Scoring (0-4 points) was based on the sum of ratings for right and left sides. The score for each hind leg, 0 = no movement, 1 = partial impairment and 2 = full movement. Values are represented as mean, n=6.

found to further enhance the blockade and extend to 40-45 min, though the peak faded around 40 min. The thermal withdrawal latencies remained considerably greater than control values even at 50 min in both P7 and in P21 rat pups that were administered with ropivacaine and clonidine or dexmedetomidine. Nevertheless, the thermal withdrawal latencies remained higher and for a longer time in pups that received ropivacaine and dexmedetomidine than ropivacaine and clonidine injection irrespective of whether administered to P7 or P21 pups (**Figure 4**). The latencies were observed to be markedly higher and longer in the pups that were given a 10 µg dose of dexmedetomidine than 3 µg dose with ropivacaine compared to rat pups that were given clonidine and ropivacaine.

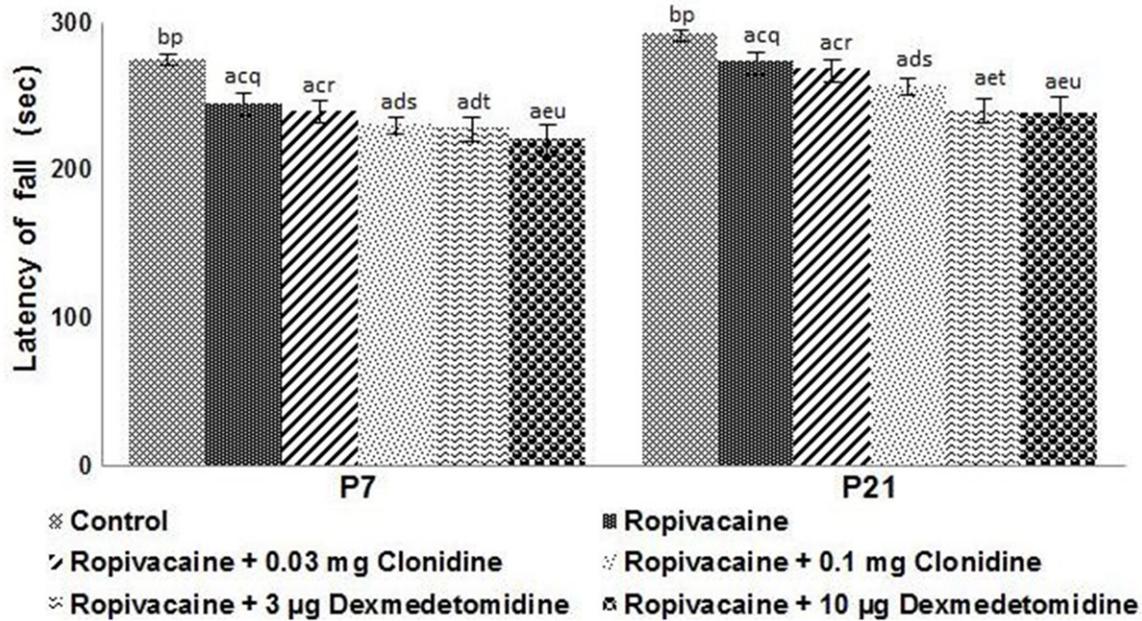
Mechanical withdrawal responses to von Frey filaments are presented in **Figure 5**. The withdrawal responses of P21 pups that received spinal anesthesia were lower in P21 pups that received no anesthesia. Similar results were observed in P7 pups that received anesthetics. The threshold values remained higher following intrathecal injections compared to control. The threshold reached a maximum between 5 to 40 min following injections in pups that received

either ropivacaine alone or in combination with dexmedetomidine or clonidine. In rat pups that received dexmedetomidine along with ropivacaine, the threshold remained higher even at 40 min as compared to pups that were exposed to clonidine with ropivacaine. Ten µg dexmedetomidine exhibited higher mechanical blocks even at 50 min as against 3 µg dose that fell from 40 min. Though marked mechanical blockade was observed on clonidine administration with ropivacaine, the results were pronounced in combined doses of dexmedetomidine and ropivacaine both in P7 and P21 rats.

### Motor block scores

Motor block scores after intrathecal injections in P7 and P21 rat pups were determined as responses to pinch with forceps. P7 and P21 pups that received neither ropivacaine nor combined doses with clonidine or dexmedetomidine showed no signs of motor impairment (**Table 1**). Ropivacaine produced dense motor block in all the pups. In all animals, the blocks recovered almost completely by 40 min. Pups that received intrathecal injections of ropivacaine along with clonidine or dexmedetomidine presented blocks even at 50 min. Pups with higher doses of clonidine or dexmedetomidine exhibited motor blocks stronger than those that received lower doses of the anesthetics (**Table 1**). Control animals that were exposed to no anesthesia responded to pinch with forceps on the skin of the back and also exhibited startled jerks and escape behaviours. All animals that received spinal injections did not exhibit any behavioural responses to pinch over the skin of the back at lumbar and lower thoracic levels, between 5-10 min after injections, however showed slight withdrawal behaviours to pinch following 30-40 min after injections. The withdrawal behaviours were not observed in animals that received ropivacaine along with clonidine or dexmedetomidine for a longer duration than pups that were administered with ropivacaine alone. The responses were observed in P21 rats before P7 rats. The combination doses with dexmedeto-

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**Figure 6.** Latency of falling of rat pups following spinal anesthesia on P7 or P21. Data are given as mean  $\pm$  SD where  $n = 3$ . <sup>a</sup>denotes statistical significance at  $P < 0.05$  related against control and <sup>b-e</sup>denotes data within the same group that differ from each other and <sup>p-u</sup>refers to statistical difference between the same treatment groups of P7 and P21 animals as calculated by one-way ANOVA followed by DMRT analysis.

midine produced denser and longer blocks than clonidine.

### Motor performance in adult rats with postnatal anesthetic exposures

Rats that were exposed to intrathecal injections either on P7 or on P21 were tested for motor performance using the rotarod apparatus following 12 h after injections. Striking differences were observed in rats that received anesthesia as against control pups that were not exposed to anesthesia though the rats that received either clonidine or dexmedetomidine along with ropivacaine exhibited falls at a much higher frequency and at shorter time intervals between the falls (**Figure 6**).

### Discussion

Short acting local anesthetics are preferred for various surgical settings; however it is becoming increasingly clear that long duration blocks are useful specifically in perioperative settings [4, 28]. One of the several strategies employed to prolong the duration of local anesthetic block is the use of adjuvant drugs which in combination with local anesthetics, can extend the duration of action. The adjunct drugs used also

have advantages of possibly reducing the dose of local anaesthetics and also reducing the toxic effects of larger doses.

Various adjuncts like epinephrine, phenylephrine, opioids or clonidine have been used to prolong the spinal anesthesia [29]. We investigated the effects of clonidine and dexmedetomidine, highly selective  $\alpha_2$ -adrenergic agonists on ropivacaine anesthesia. Previous studies have reported that dexmedetomidine has synergistic effect on ropivacaine anesthesia [30]. We investigated whether if dexmedetomidine or clonidine in combination with ropivacaine could cause a decrease in the neurotoxicity due to ropivacaine. Systemic local anaesthetic toxicity is the most dramatic manifestation of toxicity related to local anaesthetic agents [31]. In our study, ropivacaine administration resulted in a marked raise in the apoptotic cell counts in both P21 and P7 rat pups, however combined doses of ropivacaine and clonidine/dexmedetomidine caused a significant reduction in the number of TUNEL positive cells. Local anesthetics have been demonstrated to cause mitochondrial injury, caspase activation, apoptosis, increased calcium influx [32] and necrosis at higher concentrations [33].

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Antibodies to activated caspase-3 in the apoptotic cascade, identify neurons that have progressed beyond the point of commitment to cell death [34] and hence used as a marker for apoptosis. Ropivacaine spinal anesthesia resulted in a striking increase in caspase-3 positive cells in the spinal sections of rat pups. Combination doses with clonidine or dexmedetomidine markedly reduced the count of caspase-3 positive cells. Higher doses of both clonidine and dexmedetomidine were more effective in reducing the apoptotic cell counts in both P7 and P21 rat pups.

Local anesthetics and spinal analgesics are commonly co-administered to improve analgesia or reduce local anesthetic requirements [33, 35] but some analgesics enhance local anesthetic toxicity in cell culture models. Ropivacaine-induced neuronal toxicity in adult dorsal root ganglion cell culture was reported to be potentiated by midazolam [1]. Midazolam and ketamine positively influenced lidocaine toxicity in human neuroblastoma and rat astrocyte cultures, whereas sufentanil, clonidine, epinephrine and neostigmine had no effects on lidocaine induced toxicity [36]. In our study, clonidine and dexmedetomidine did not enhance the decrease in cell viability due to ropivacaine anesthesia. Further, ropivacaine combination with either clonidine or dexmedetomidine resulted in a marked decrease in apoptotic cell counts as well markedly normalized glial reactivity markers GFAP and Iba1, indicating that clonidine and dexmedetomidine effectively offered protection to neurons.

Local anesthetics may have additional toxic effects unrelated to developmental apoptosis that influence both motor and sensory outcomes. In adult animals, changes in thermal tail flick latency, mechanical paw pressure withdrawal threshold, and motor function have been demonstrated to occur in about 4 to 7 days following doses of local anesthetics that cause tissue damage [37-40].

Responses of the rat pups to thermal and mechanical stimulus were assessed using hot plate and von Frey filaments. We observed single dose of intrathecal ropivacaine at 0.5% producing reliable sensory and motor blockade in rat pups at P7 and P21. The results of the assessment of sensory and motor blocks in rat

pups following intrathecal anaesthesia of ropivacaine suggested that combined dose of ropivacaine and dexmedetomidine could induce sensory and motor blocks for a longer duration than ropivacaine along with clonidine. Further administration of dexmedetomidine at a higher concentration with ropivacaine resulted in stronger blocks.

To further analyze the extent of blockade, the rats were subjected to rotary movement on a rotarod apparatus. The apparatus determines the capacity of the rats in rotating a spindle as a measure of motor performance. The rats were observed at 12 h following anesthesia. The latency of fall was recorded as the efficiency of the rats in rotating the spindle. The rats exposed to intrathecal anesthesia of ropivacaine presented shorter period of fall. Rats that received clonidine or dexmedetomidine along with ropivacaine had much shorter periods of fall. The results of rotation of the spindle indicate that clonidine and dexmedetomidine brought about stronger blocks than when ropivacaine is administered as a single drug.

The mechanisms by which intrathecal  $\alpha_2$ -adrenoceptor agonists prolong the motor and sensory block of local anesthetics is not well understood. Yaksh [41] reported that intrathecal  $\alpha_2$ -adrenoceptor agonists can cause a dose-dependent decrease in motor strength in animals. The prolongation of the motor block of spinal anesthetics may have possibly resulted from the binding of  $\alpha_2$ -adrenoceptor agonists to motor neurons in the dorsal horn [42].

Previous studies have suggested a 1:10 dose ratio between intrathecal dexmedetomidine and clonidine producing a similar effect in animal models [43, 44]. Further the potency of epidurally administered  $\alpha_2$ -adrenoceptor agonists correlated with their binding affinity to spinal  $\alpha_2$ -adrenoreceptors [44]. The binding affinity of dexmedetomidine compared with clonidine is approximately 1:10 [44]. Thus in our study, the doses that were selected for dexmedetomidine and clonidine combination were in the ratio 1:10. However, dexmedetomidine given along with ropivacaine was observed to be more effective not only in prolonging the anesthetic effects but also in reducing the neurotoxic effects of ropivacaine when administered alone.

## Conclusion

Our study attempted to evaluate the anesthetic effects of dexmedetomidine and clonidine in combination with ropivacaine in neonatal rats. The observations are suggestive of the potency of drug combination in extending the duration and strength of spinal anesthesia and as well on reducing neurotoxic effects associated with ropivacaine anesthesia.

## Disclosure of conflict of interest

None.

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