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# Dexmedetomidine promotes cell proliferation and attenuates insulin resistance in high glucose induced trophoblast cells via inactivating the p38 MAPK pathway

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**ABSTRACT**: Gestational diabetes mellitus (GDM) is a disease that occurs during pregnancy. Promoting the proliferation and migration of trophoblast cells and increasing insulin sensitivity are the strategies to improve GDM. Dexmedetomidine (DEX) is a highly selective α 2-adrenoceptor agonist with sedative and analgesic effects and has been widely used in clinical anesthesia. However, the exact effect of DEX on GDM is still unclear. In this study, the effects of DEX on the progression of GDM were investigated. A high glucose (HG) cell model was constructed via treating Human trophoblast cell line HTR-8/SVneo with 25 mmol/l glucose. The results showed that DEX promoted HTR-8/SVneo cell proliferation of high glucose induced cells and suppressed the apoptosis. In addition, DEX promoted insulin sensitivity in hTR-8 /SVneo cells induced by high glucose. Mechanically, DEX suppressed the p38 MAPK pathway in hTR-8/SVneo cells, resulting in stimulation of cell proliferation and suppression of insulin resistance. In conclusion, these results suggested that DEX promotes cell proliferation and attenuates insulin resistance in GDM.

**KEYWORDS**: gestational diabetes mellitus (GDM), dexmedetomidine (DEX), cell proliferation, insulin resistance, p38 MAPK pathway

## INTRODUCTION

Gestational diabetes mellitus (GDM) is a disease that occurs during pregnancy [1]. If GDM is not treated in time, it can cause a series of serious consequences, such as abnormal fetal development, miscarriage, and fetal asphyxia [2]. GDM may lead to placenta dysplasia and incorrect vascular remodeling. The placenta is an important organ for the exchange of nutrients between mother and fetus during pregnancy. Studies have shown that invasion, migration, and activity of trophoblast cells induced by high glucose may lead to placental dysplasia, resulting in abortion, spontaneous abortion, and premature delivery. Therefore, promoting the function of trophoblast cells and increasing insulin sensitivity are the ideas to improve GDM.

Dexmedetomidine (DEX) is a highly selective α 2-adrenoceptor agonist with sedative and analgesic effects and has been widely used in clinical anesthesia in recent years [3]. In addition, DEX has been found to have a protective effect on organs [4]. For example, DEX inhibits inflammation and oxidative stress induced by ischemia-reperfusion and alleviates injury by activating the PI3K/AKT pathway [5, 6].

DEX can reduce insulin resistance in liver cells by reducing endoplasmic reticulum stress, and it can also reduce blood glucose and restore insulin action by activating PI3K/AKT pathway [7]. In addition, DEX plays an important role in maintaining normal pregnancy. DEX preconditioning decreased the expression of COX-2 and PGE2, and attenuated the release of lPS-induced inflammatory factor [8]. Numerous studies have found that DEX can inhibit the activity of MAPK [9], resulting in reduction of insulin resistance. However, the exact effect of DEX on GDM is still unknown.

In this study, the effects of DEX on the progression of GDM were investigated using a cell model. The results showed that DEX promoted the proliferation of trophoblast cells induced by high glucose and inhibited insulin resistance by inhibiting the p38 MAPK pathway.

## MATERIALS AND METHODS

### Cell culture and treatment

Human trophoblast cell line HTR-8/SVneo, purchased from BeNa Cultrue Collection (Beijing, China), was cultured in DMEM/F12 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin in 5% $CO_2$ hood at 37 °C. Cells were incubated with a series of glucose concentrations (5.5, 12.5, 25, and 50 mM) for 72 h. The cells were, then, treated with DEX of 0, 0.25, 0.5, 1 and 2 nM concentrations for 24 h to be used in further experiments.

### Cell viability

The DEX treated HTR-8/SVneo cells were transferred to 96-well plates at the density of $3 \times 10^3$ cells/well. Cell viability was determined with the addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT).

### EdU staining

The DEX treated HTR-8/SVneo cells were fixed with formaldehyde, rinsed with TBS and then stained with EdU staining kit (Abcam, Cambridge, UK).

### Lactate dehydrogenase release

The experiment was performed on the DEX treated HTR-8/SVneo cells. Lactate dehydrogenase (LDH) Cytotoxicity Assay Kit (Thermo Fisher Scientific, USA) was used for the detection of cellular LDH release. Briefly, the medium of each well was collected and added into a 96-well flat-bottom plate, and reaction mixture was added to the samples. After incubation for 30 min under dark and addition of Stop Solution, the absorbance in each well was determined using Microplate Reader (Molecular Devices, Germany).

### Cell apoptosis

The experiment was performed using the DEX treated HTR-8/SVneo cells. Annexin V/PI apoptosis detection kit was used for the measurement of apoptotic cell number in groups in accordance with manufacturer's protocol ((Sigma Aldrich, USA) and previous study [10]. Briefly, cells were digested and resuspended in binding buffer containing Annexin V and PI for 5 min at room temperature. Cell apoptosis was determined by a flow cytometer (BD Biosciences, USA).

### Immunoblot assay

Proteins were extracted with RIPA buffer (Beyotime, Beijing, China). Then, the samples were collected and electrophoresed by 9% SDS-PAGE, and transferred onto PVDF membranes, followed by blocking with 5% fat-free milk. Subsequently, membranes were incubated with primary antibodies for 2 h and, then, conjugated with specific secondary antibodies for 1 h. The blots were analyzed using ECL kit.

### Glucose uptake

The DEX treated HTR-8/SVneo cells were rinsed with PBS and dispensed with DMEM containing 10 mM glucose for 10 min at 37 °C. Then, cells were put on ice. Each well was incubated with cold PBS and Inactivation Solution (0.6 N HCl) for 5 min on a plate stirrer and Neutralization Solution (Tris Base 1 M) for 30–60 s. The intracellular glucose level was detected after incubation with Amplex Red Glucose Assay Kit® (Thermo Fisher Scientific, USA) for 30 min covered by light. The reaction was monitored by assessing at 530–560 nm.

### Statistical analysis

Data were presented as mean ± SD. Statistical analysis was performed using GraphPad. $p < 0.05$ was considered as statistically significant.

## RESULTS

### DEX promotes cell proliferation induced by HG in HTR-8/SVneo cells

To evaluate the effect of HG on cell viability, HTR-8/SVneo cells were incubated with increasing concentrations of glucose. As shown in Fig. 1A, cell viability in HTR-8/SVneo cells was significantly impaired, reflected with the decreased absorbance at 490 nm and confirmed by MTT assays. The effect of DEX on cell viability was further measured by MTT assay. Results showed that cell viability was affected by DEX at 1 and 2 nM, with the obvious change of absorbance at 490 nm (Fig. 1B). HG treatment led to decrease in cell viability of HTR-8/SVneo. DEX treatment improved cell viability of HG stimulated cells, as shown by the increase of absorbance at 490 nm (Fig. 1C). Moreover, cell proliferation of the DEX treated HTR-8/SVneo cells was measured by EdU staining (Fig. 1D). HG inhibited cell proliferation, and DEX restored cell proliferation, with the increased Edu-positive cell numbers (Fig. 1E). LDH level was enhanced in HG and reduced by DEX (Fig. 1F). Collectively, DEX improves HTR-8/SVneo cell proliferation in HG medium.

### DEX restrains HG-induced cell apoptosis in HTR-8/SVneo cells

Cell apoptosis in response to DEX and HG was determined by Flow cytometry. As shown in Fig. 2A, HG treatment enhanced cell apoptosis, and DEX relieved cell apoptosis in HTR-9/SVneo cells, as seen by the increased percentage of apoptosis cells (Fig. 2A,B). Moreover, while the levels of Bax, cleaved caspase-3, and cleaved caspase-9 were increased by HG, the Bcl-2 level was decreased. DEX treatment reversed the expression of these proteins (Fig. 2C). In overall, DEX restrains HG-induced cell apoptosis in HTR-8/SVneo cells.

### DEX promotes insulin sensitivity in HG treated hTR-8 /SVneo cells

The insulin sensitivity in DEX treated cells was determined. HG treatment reduced GLUT4 and p-IRS-1(Tyr896) levels and contrarily enhanced p-IRS-1(Ser307) level. DEX treatment restored the alterations induced by HG (Fig. 3A). The glucose uptake was impaired by HG, and DEX significantly improved glucose uptake (Fig. 3B). Therefore, DEX promotes insulin sensitivity in HG treated hTR-8/SVneo cells.

### DEX promotes proliferation of trophoblast cells induced by HG and inhibits insulin resistance by inhibiting the p38 MAPK pathway

To reveal the related mechanisms underlying the role of DEX in cell proliferation and insulin sensitivity, the MAPK pathway was examined. The elevated level of p-ERK, p-JNK, and p-p38 in HG-induced trophoblast cells was observed. DEX significantly alleviated the accumulation of p-ERK, p-JNK, and p-p38 (Fig. 4). These results indicate that DEX promotes the proliferation of trophoblast cells induced by HG and inhibits insulin resistance by inhibiting the p38 MAPK pathway.

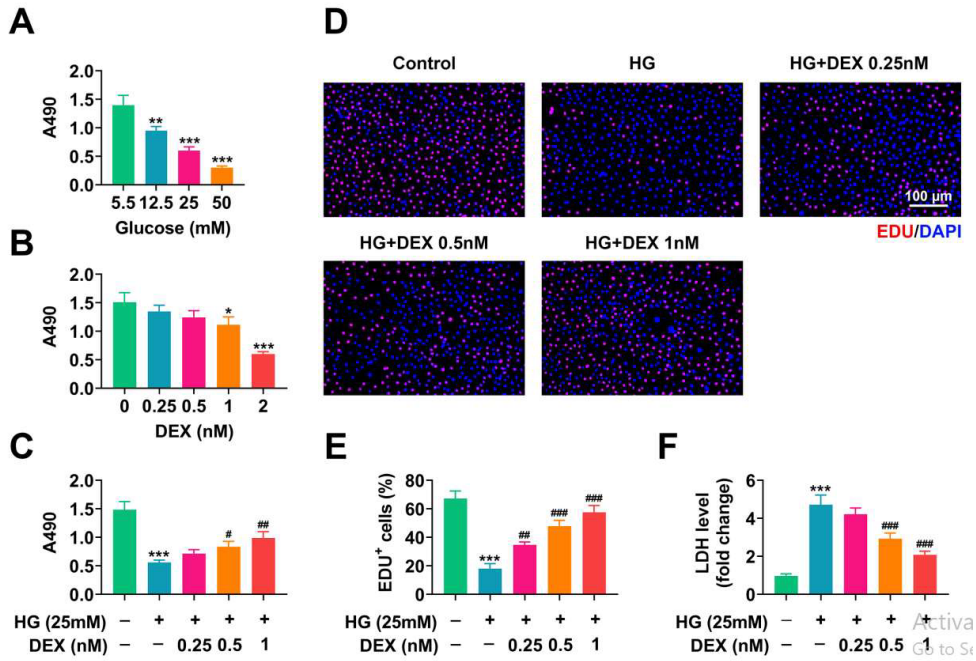


**Fig. 1** DEX promotes HG-induced cell proliferation in HTR-8/SVneo cells. (A) Cell viability in response to glucose concentrations; (B) cell viability in response to DEX levels; (C) HG treated cell viability in response DEX levels detected by MTT assay; (D,E) HG treated cell proliferation in response to DEX levels detected by EdU staining; (F) LDH level in response to HG and DEX levels. All the experiments in this figure were repeated 3 times. Data are presented as mean ± SEM, $^{***}p < 0.001$ vs. NG, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. HG.

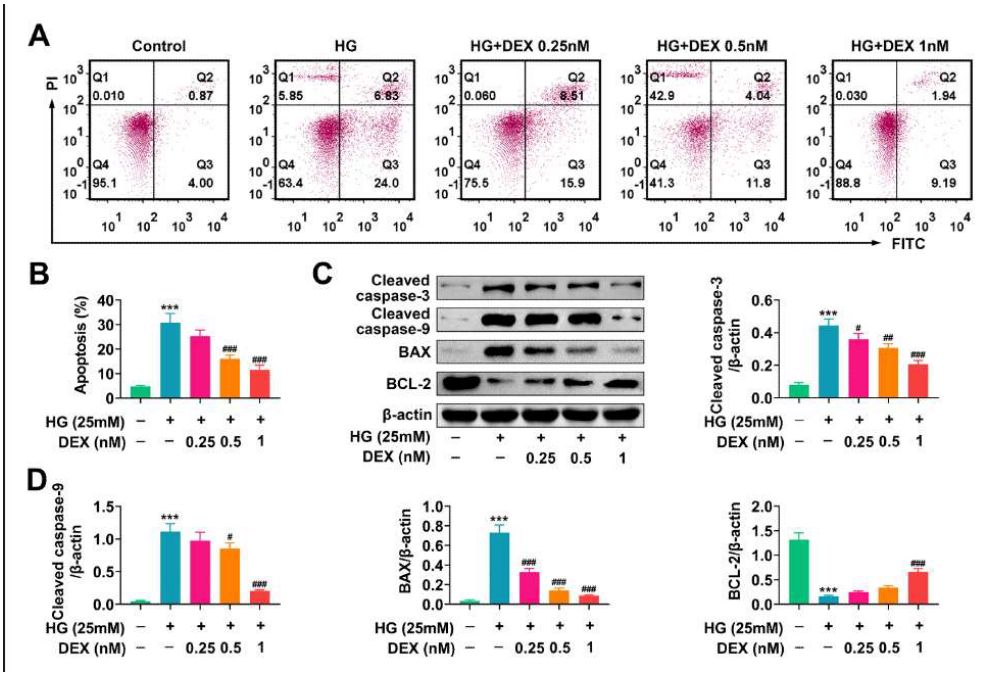


**Fig. 2** DEX restrains HG-induced cell apoptosis in HTR-8/SVneo cells. (A,B) Cell apoptosis detected by Flow cytometry; (C,D) the level of cleaved caspase-3, cleaved caspase-9, Bax, and Bcl-2 in response to DEX levels. All the experiments in this figure were repeated 3 times. Data are presented as mean ± SEM, $^{***}p < 0.001$ vs. NG, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. HG.

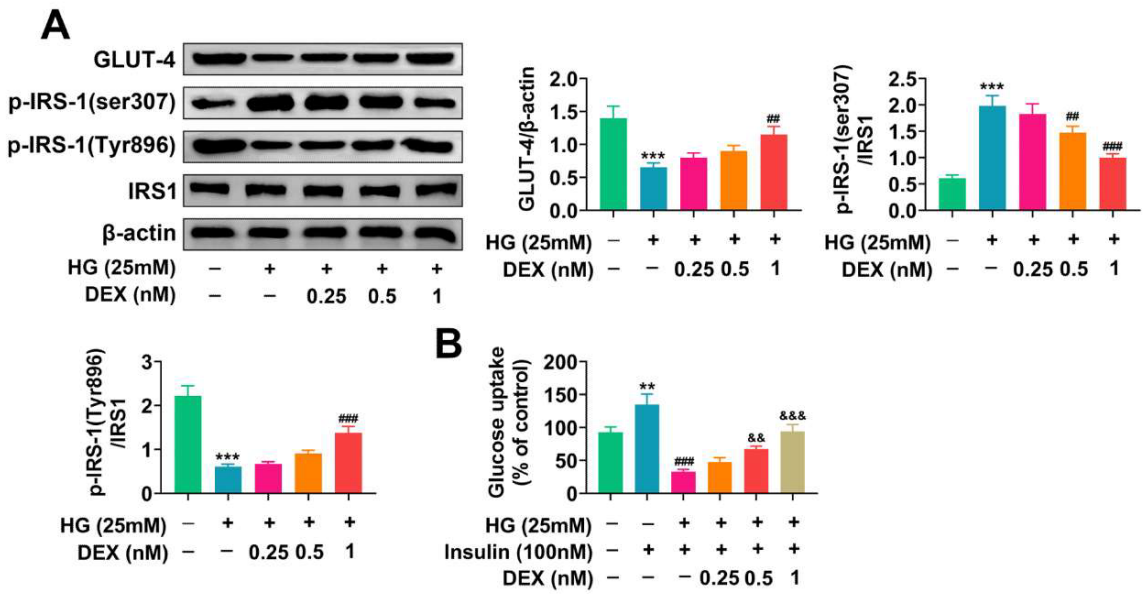


**Fig. 3** DEX promotes insulin sensitivity in hTR-8 /SVneo cells induced by HG. (A) Levels of GLUT-4, p-IRS (ser307), p-IRS(tyr896) in response to DEX levels; (B) glucose uptake in response to insulin and DEX levels. All the experiments in this figure were repeated for 3 times. Data are presented as mean ± SEM, $^{***}p < 0.001$ vs. NG, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. HG.

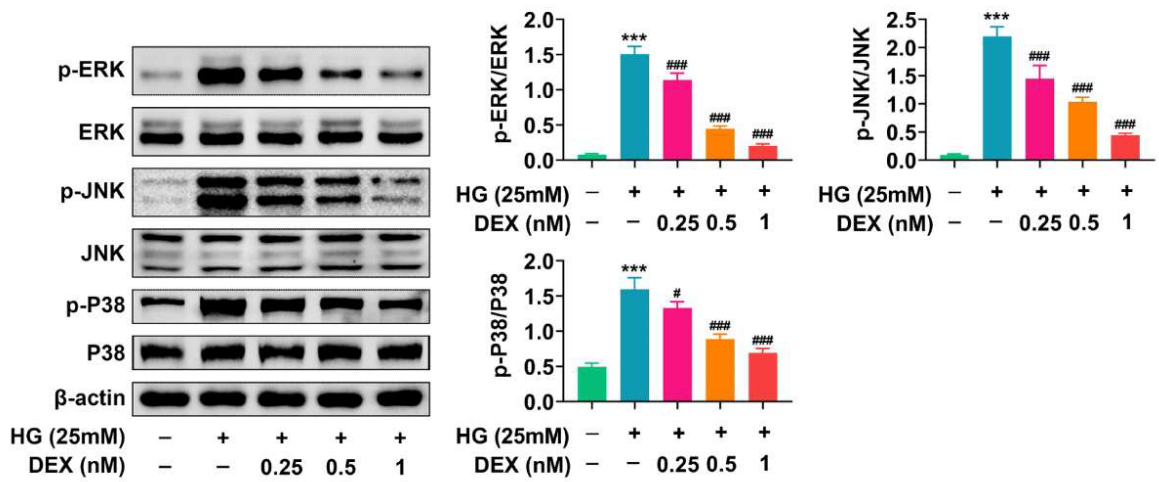


**Fig. 4** DEX promotes the proliferation of trophoblast cells induced by HG and inhibits insulin resistance by inhibiting the p38 MAPK pathway. Immunoblot assays depicted the expressions of p-ERK, p-JNK, and p-p38 in HG-induced hTR-8 /SVneo cells. All the experiments in this figure were repeated 3 times. Data are presented as mean ± SEM, $^{***}p < 0.001$ vs. NG, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. HG.

## DISCUSSION

The incidence rate of GDM is 1%–14% in the world and 1%–5% in China, which has significantly increased in recent years. In order to improve the intervention of GDM, further research on its pathogenesis is urgently needed [11]. Inhibiting invasion, migration, and activity of trophoblast cells induced by HG may lead to placental dysplasia, resulting in abortion, spontaneous abortion and premature delivery due to GDM [12]. Therefore, promoting the proliferation and migration of trophoblast cells and increasing insulin sensitivity

are the ways to improve GDM. Interestingly, DEX had the potential to serve as a drug in GDM treatment. Our data confirmed that DEX promoted the HG induced-cell proliferation and attenuated insulin resistance.

The effects of DEX on the proliferation and apoptosis of HG induced trophoblast cells were revealed by performing CCK-8, Edu, and FCM assays. The additional results indicated that DEX promotes insulin sensitivity in HG induced hTR-8/SVneo cells. Therefore, the effects of DEX on GDM *in vitro* were investigated. In this study, the treatment concentration of DEX was set according to the previous study, and we confirmed its effects on HG-induced cells [13]. The effects of DEX on the progression of several diseases have been widely revealed [13]. Recently, serum carbonic anhydrase combined with adenopectin have been reported as the useful biomarkers in the diagnosis of insulin resistance in human [14]. For example, DEX suppressed serum syndecan-1 upregulation and improved survival in a rat hemorrhagic shock model [15]. In addition, DEX promoted apoptosis and suppressed proliferation of hepatocellular carcinoma (HCC) cells via mediating the microRNA-130a/EGR1 axis [16]. Similarly, our study showed that DEX affected proliferation and apoptosis of HG induced hTR-8 /SVneo cells. DEX could also reduce the oxidative stress and serum miR-10a levels in lung cancer patients. These studies all confirmed the effects of DEX on different types of diseases.

In fact, the role of DEX in metabolic diseases has been uncovered. Dynamics of heart rate variability in patients with diabetes during spinal anesthesia using DEX has been disclosed [17]. In addition, the effect of DEX on intraoperative blood glucose homeostasis has been reported. Importantly, a previous study indicated the protective effect of DEX against renal injury in diabetic nephropathy rats via NF-κB pathway [18]. However, results of the present study showed that DEX promoted the HG induced cell proliferation and attenuated insulin resistance via p38 MAPK pathway. Moreover, DEX has been found to have a protective effect on organs, e.g., inhibition of inflammation and oxidative stress induced by ischemia-reperfusion and alleviation of injury by activating the PI3K/AKT pathway. DEX was shown to reduce cardiac dysfunction and autophagy damages in diabetic rats by promoting AKT phosphorylation and reducing ERK phosphorylation. Therefore, DEX could be used as a potential drug for the treatment of diabetes-related diseases.

The p38 MAPK pathway is critical in the metabolic regulation. Several studies confirmed its role in GDM progression, and multiple drugs suppressed the progression of GDM via this pathway [19]. Our study proved that DEX promoted the HG induced cell proliferation and attenuated insulin resistance via this pathway. Therefore, the p38 MAPK pathway could serve as a target for GDM treatment.

In conclusion, our study shows that DEX promotes the proliferation and suppresses the apoptosis of HG-induced trophoblast cells, and promotes insulin sensitivity through mediating the p38 MAPK pathway.

## REFERENCES


1. Rahnemaei FA, Pakzad R, Amirian A, Pakzad I, Abdi F (2022) Effect of gestational diabetes mellitus on lipid profile: A systematic review and meta-analysis. *Open Med* **17**, 70–86.
2. Adesina N, Dogan H, Green S, Tsofliou F (2021) Effectiveness and usability of digital tools to support dietary self-management of gestational diabetes mellitus: A systematic review. *Nutrients* **14**, 10.
3. Zhou L, Xie Q, Zhang Q, Hu M, Ma T, Xie H (2022) Dexmedetomidine attenuates motor deficits via restoring the function of neurons in the nigrostriatal circuit in Parkinson's disease model mice. *Eur J Pharmacol* **920**, 174806.
4. Yang CJ, Chiu CT, Yeh YC, Chao A (2022) Successful management of delirium with dexmedetomidine in a patient with haloperidol-induced neuroleptic malignant syndrome: A case report. *World J Clin Cases* **10**, 625–630.
5. Tang Y, Liu J, Huang X, Ding H, Tan S, Zhu Y (2021) Effect of dexmedetomidine-assisted intravenous inhalation combined anesthesia on cerebral oxygen metabolism and serum Th1/Th2 level in elderly colorectal cancer patients. *Front Surg* **8**, 832646.
6. Rudikoff AG, Tieu DD, Banzali FM, Nguyen CV, Rettig RL, Nashed MM, Mora-Marquez J, Chen Q, et al (2022) Perioperative acetaminophen and dexmedetomidine eliminate post-operative opioid requirement following pediatric tonsillectomy. *J Clin Med* **11**, 561.
7. Nasreen F, Athar M, Khalid A, Mallur DS (2021) A Randomised controlled trial to assess the analgesic efficacy of reduced dose 0.2% ropivacaine-dexmedetomidine combination compared to standard 0.375% ropivacaine in USG guided TAP block for paediatric hernia repair. *Turk J Anaesthesiol Reanim* **49**, 304–311.
8. Hu J, Zhang Y, Maze M (2022) Dexmedetomidine for prevention of postoperative delirium in older adults undergoing oesophagectomy with total intravenous anaesthesia. *Eur J Anaesth* **39**, 296.
9. Guo H, Ao T, Wang J, Zhang X, Zheng J, Xiao Y, Xue R, Kalika P, et al (2022) Clinical efficacy of perioperative intravenous dexmedetomidine and lidocaine combined infusion for thyroidectomy: A prospective, randomized, double-blind, placebo-controlled trail. *Clin J Pain* **38**, 264–270.
10. Alhasan L, Addai Z (2018) Allicin-induced modulation of angiogenesis in lung cancer cells (A549). *Trop J Pharm Res* **17**, 2129–2134.
11. Doust JA, Glasziou PP, d'Emden MC (2022) A large trial of screening for gestational diabetes mellitus in the United States highlights the need to revisit the Australian diagnostic criteria. *Med J Aust* **216**, 113–115.
12. Du Y, Rafferty AR, McAuliffe FM, Wei L, Mooney C (2022) An explainable machine learning-based clinical decision support system for prediction of gestational diabetes mellitus. *Sci Rep* **12**, 1170.

13. Cote E, Zwicker LA, Anderson EL, Stryhn H, Yu J, Andersen E (2022) Effects of dexmedetomidine and its reversal with atipamezole on echocardiographic measurements and circulating cardiac biomarker concentrations in normal cats. *J Am Vet Med Assoc* **260**, 1–9.
14. Piumngam K, Siriprungpong P, Roytrakul S (2021) Serum carbonic anhydrase combined with adiponectin as biomarkers of insulin resistance. *ScienceAsia* **47**, 287–292.
15. Cheng YT, Lee KT, Chang CH, Wu VC, Chan YS, Chen DY, Chu PH, Chou AH, et al (2022) Effects of dexmedetomidine on surgery for type A acute aortic dissection outcome. *Sci Rep* **12**, 2761.
16. Zhou L, Li J, Liu X, Tang Y, Li T, Deng H, Chen J, Yin X, et al (2022) Dexmedetomidine promotes apoptosis and suppresses proliferation of hepatocellular carcinoma cells via microRNA-130a/EGR1 axis. *Cell Death Discov* **8**, 31.
17. Xiao R, Liu LF, Luo YR, Liu C, Jin XB, Zhou W, Xu GH (2022) Dexmedetomidine combined with femoral nerve block provides effective analgesia similar to femoral nerve combined with sciatic nerve block in patients undergoing total knee arthroplasty: A randomized controlled study. *Drug Des Dev Ther* **16**, 155–164.
18. Wang J, Li Y, Xiao S, Shi B, Xia Z, Huang C, Xu H, Li N, et al (2022) Efficacy and safety of intranasal dexmedetomidine versus oral chloral hydrate as sedatives for pediatric patients: A systematic review and meta-analysis. *J Investig Med* **70**, 1219–1224.
19. El Sherif FA, Abdel-Ghaffar H, Othman A, Mohamed S, Omran M, Shouman S, Hassan N, Allam A, et al (2022) Pharmacokinetics and pharmacodynamics of dexmedetomidine administered as an adjunct to bupivacaine for transversus abdominis plane block in patients undergoing lower abdominal cancer surgery. *J Pain Res* **15**, 1–12.