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Research article

Network pharmacology of xylazine to understand its health consequences and develop mechanistic-based remediations

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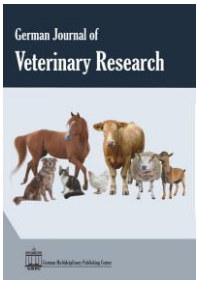

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Abstract

The recent rise in xylazine use disorders (XUD) in humans is a significant cause for concern as a comprehensive understanding of its molecular pathology is limited, and hence, the ability to reverse the potential adverse effects is lacking. To address this gap, this study evaluates the dose-dependent impact of xylazine and its interactions with various potential targets to identify an optimal reversal strategy. A trichotomized (Low, medium, and high) dose, volume of distribution, and predicted plasma concentration of xylazine were defined. A detailed analysis of xylazine's network protein targets and their tissue-specific expression was performed using classical pharmacoinformatic tools. Molecular docking was used to assess the drug-target affinities and identify potential reversal agents. The study categorized xylazine plasma concentrations ranging from 5-8 µM, 14-20 µM, and 28-40 µM as low, medium, and high concentrations, respectively. Xylazine displayed a preferential affinity for hydrolases, kinases, transporters, and ion channels. Xylazine's network analysis revealed the following proteins: ABCC9, RET, RAPGEF4, ACHE, TGFBR1, PGR, KCNH2, KCNN2, and TRPM8 as its high-affinity targets. The tissue-specific expression of these high-affinity targets suggested potential adverse effects on various organs, particularly skeletal and smooth muscles and the adrenal gland. The study further explored the potential reversal of xylazine pharmacology using alpha2AR-antagonists and CNS stimulants. Prazosin emerged as the most promising candidate, exhibiting a 200 to 2000-fold superior affinity against all high-affinity targets of xylazine. This study contributes to our understanding of xylazine's molecular mechanisms, which could be relevant to its pharmacological effects in all species, and suggests that prazosin can serve as an effective therapeutic option for mitigating xylazine-induced adverse effects in XUD patients, which warrants clinical investigation.

Keywords: Drug abuse, Prazosin, Toxicity, Xylazine, Xylazine use disorders

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Introduction

Xylazine is an alpha-2 adrenergic receptor agonist and is approved by the Food and Drug Administration (FDA) for veterinary use as a pre-anesthetic agent (Hovda et al., 2011; Ball et al., 2022; Malaca et al., 2023). The pharmacological effects of xylazine are mediated by the activation of alpha-2 adrenergic receptors in the central nervous system (CNS), leading to sedation, muscle relaxation, and analgesia in animals, and is not approved by the FDA for use in humans. Unfortunately, xylazine is adulterated with illicit drugs (fentanyl, cocaine) either to amplify the effects of the drugs or to boost the street value of illicit drugs by adding weight. The prevalence of xylazine co-use with opioid/stimulant combinations in humans is widely reported to be responsible for serious life-threatening effects (respiratory depression, hypotension, bradycardia vasoconstriction, and severe necrotic skin ulcerations/infections) leading to significant morbidity and mortality (Rubin, 2022; Ayub et al., 2023; Pergolizzi Jr et al., 2023; Sibbesen et al., 2023).

Currently, the mechanistic insights into the undesired effects produced by misuse of xylazine are lacking (Quijano et al., 2023; Zagorski et al., 2023), and therefore, there are not any FDA-approved agents to reverse xylazine adverse effects in humans. Additionally, the off-label use

of adrenergic antagonists or modulators for emergency treatment of accidental xylazine injection has not shown acceptable clinical efficacy (D'Orazio et al., 2023; Malaca et al., 2023; Sibbesen et al., 2023; Zagorski et al., 2023). Following an extensive review of the impact of xylazine on the opioid crisis and its growing role in overdose deaths in all regions of the United States, the White House's Office of National Drug Control Policy has recently designated fentanyl mixed with xylazine as an emerging public health threat to the United States (Kariisa, 2023; Rubin, 2023).

Limited information is available regarding the consequences of using drugs adulterated with xylazine and the mechanisms by which the observed undesired effects or potential substance use disorders (SUDs) are produced. Research into the consequences of xylazine use, which leads to undesired effects and impacts the treatment of opioid abuse and overdose, is necessary to develop mechanistic-based treatment of opioid abuse, and overdose is necessary to develop mechanistic-based optimal clinical interventions. To address this gap in the literature, in this study, the network pharmacology of xylazine was evaluated to know the receptor binding profiles of xylazine and identify suitable antagonists with the potential to reverse xylazine pharmacology.

Materials and methods

Structure of xylazine and identifying its networks

The isomeric SMILES sequence of xylazine (CC1=C(C(=CC=C1)C)NC2=NCCCS2) obtained from the PubChem database was inputted into the Swiss Target Prediction server (http://www.swisstargetprediction.ch/) and STITCH database (http://stitch-db.org/) to identify the targets specific to homo sapiens (Friend and Kumar 2023; Singh NK et al., 2023; Kumar, 2024). Briefly, the SMILES sequence of xylazine was inputted into the box allocated for drawing the chemical structure, the "homo sapiens" option was selected from the species option available, and following this, the "Predict target" button was pressed to get the list of xylazine targets. Similarly, in the STITCH database, the SMILES sequence of xylazine was inputted into the box allocated for this purpose, the homo sapiens option was selected from the drop-down menu, and the search tool was clicked to retrieve the top networks of xylazine.

Pharmacokinetic parameters of xylazine

The pharmacokinetic parameters of xylazine were assessed using the SwissADME server and data reported in the literature. The observed dosage (mg/day), plasma concentration achieved (μM), and volume of distribution (L/Kg) were trichotomized into low, medium, and high categories based on specific percentiles (lower 33%, middle 34%, upper 33%).

Molecular docking of xylazine and its targets

The affinity values of xylazine to all its potential targets identified were assessed using Auto Dock vina 1.2.0, as reported before, for other ligand-receptor combinations (Friend and Kumar, 2023; Khosravi and Kumar, 2023). The observed targets of xylazine were categorized based on various functional groups reported in the output files from the Swiss Target Prediction server search reported above, and the average affinity of xylazine towards each of the functional groups was estimated to assess the selectiveness of xylazine to any specific target group/s.

The high-affinity targets of xylazine were identified based on a concentration affinity (CA) ratio of >0.077. The specific interaction sites, binding pocket sequence, EC_{50} values, and the number of hydrogen bonds formed during receptor-ligand interaction were also estimated, as reported previously (Friend and Kumar, 2023; Singh et al., 2023).

Expression pattern and function of the high-affinity targets of xylazine

The tissue-specific expression pattern of the high-affinity targets of xylazine was assessed using the human protein atlas (https://www.proteinatlas.org/) database. The gene code of the high-affinity target was inputted into the search tool of the human protein atlas database, and the "tissue" tool was used to extract the protein (or mRNA) expression pattern of the target in various tissues. Only the major expression (top three tissues expressing medium/high levels of the target) was of focus in this study and are presented as Venn diagrams. The primary function of all the high-affinity targets was identified from the UniProt (https://www.uniprot.org/) database.

Antagonize the xylazine pharmacology

To identify the most optimal approach to

antagonize the xylazine pharmacology, in this study, most known alpha-2 adrenergic receptor-modulators (yohimbine, chlorpromazine, phentolamine, mianserine, spiperone, prazosin, alprenolol, propranolol, pindolol, atipamezole, dexmedetomidine, and tolazoline) and CNS stimulants (4-aminopyridine, doxapram, and caffeine) which are approved for clinical use were assessed for their affinity against all high-affinity targets of xylazine as mentioned above (Friend and Kumar 2023; Singh et al., 2023; Kumar, 2024) A heat map of the affinity ratio values of the agonist (xylazine) / antagonist was generated to identify the most optimal drug to antagonize the xylazine pharmacology.

Results

Xylazine consists of the 2,6-dimethylphenylamino group, which is attached to the fourth carbon atom of the thiazine ring (Figure 1A). The nitrogen and sulfur atoms in the thiazine ring and the 2,6-dimethyl phenylamino group seem to be the key components responsible for xylazine's pharmacological properties, particularly in its interaction with alpha-2 adrenergic receptors. In this study, we defined the dose of xylazine into the following three categories: 1) low (<300 mg/day, observed range 150 to 300 mg/day), 2) medium (>300 and <800 mg/day) and 3) high (>800 mg/day, observed range 800 to 1200 mg/day) (Figure 1B). These dose ranges were used to estimate the plasma concentrations achievable in humans, which ranged from 5-8 µM (low dose), 14-20 µM (medium dose), and 28-40 µM (high dose) by considering the following variable volume of distribution of xylazine in humans [1.9 to 2.0 liters/Kg (low), 2.25 to 2.42 liters/Kg (medium) and 2.58 to 2.75 liters/kg (high)] (Figure 1B).

The xylazine network analysis in the STITCH database showed six network proteins, of which four were close networks (affinity scores >0.8) consisting of alpha-2 adrenergic receptors (types 2A, 2B and 2C) and RAS (RAD and GEM)-like GTP-binding 1 (REM1), while the other two proteins showed weaker network (affinity scores <0.5), consisting of hydroxysteroid (17-beta) dehydrogenase 12 (HSD17B12) and translocator protein (TSPO) (Figure 1C). The network analysis of xylazine in the Swiss Target Prediction server showed 105 potential targets (Figure 1D). The affinity of the xylazine to these targets ranged from 147 to 10616 µM). The following nine targets, ABCC9, RET, RAPGEF4, ACHE, TGFBR1, PGR, KCNH2, KCNN2, and TRPM8, had affinity values of <500 µM with xylazine and were also recognized as high-affinity targets based on their concentration affinity (CA) ratio of <0.077 (Figure 1D). The highest affinity of xylazine was observed for ABCC9 (147 µM). To assess if xylazine has a preferential affinity to any specific target class, the 105 potential targets were grouped into 12 target classes, and the average affinity of xylazine to each of the target classes was estimated and summarised in Figure 1. The highest affinity of xylazine was observed for hydrolases, kinases, transporters, and ion channels, this was followed by affinity for Family A GPCR and cytochrome P_{450} (Figure 1E). The least affinity of xylazine was observed for oxidoreductases (Figure 1E).

Incidentally, the alpha-2 adrenergic receptors (ADRA2A, ADRA2B, and ADRA2C) were not among the high-affinity targets of xylazine, with affinity values ranging from 1590 to 1921 µM. Hence EC_{50} values (extrapolated from the binding energies) of xylazine against all its high affinity targets and the alpha-2 adrenergic receptors were evaluated (Figure 2A). The least EC_{50} of xylazine was observed for PGR, followed by TRPM8 and ADRA2A (Figure 2B). The specific binding sequence of xylazine targets identified in this study, together with the number of hydrogen bonds formed during the interaction, are summarised in Figure 2C. The correlation between the EC_{50} values and the number of hydrogen bonds formed between xylazine and its high-affinity targets suggested a very weak correlation (r^2<0.016) (data not shown). The weak correlation between binding affinity and hydrogen bonding pattern is consistent with the intricate nature of ligand-receptor interactions. The highest number of hydrogen bonds (>18) between xylazine and its high-affinity targets was observed for TGFBR1, ADRA2A, ABCC9, ADRA2B, and ACHE (Figure 2C). At the same time, the number of hydrogen bonds formed between xylazine and RAPGEF4, PGR, TRPM8, and ADRA2C ranged from 13-15 bonds. RET, KCNH2, and KCNN2 showed the least number (<10) of hydrogen bonds formed with xylazine (Figure 2C).

The tissue-specific expression of all the high-affinity targets of xylazine was assessed from the human protein atlas database and is summarised in Figure 3A. The targets of xylazine, which formed the most number of hydrogen bonds (>18), were mainly expressed in bone marrow,

Figure 1

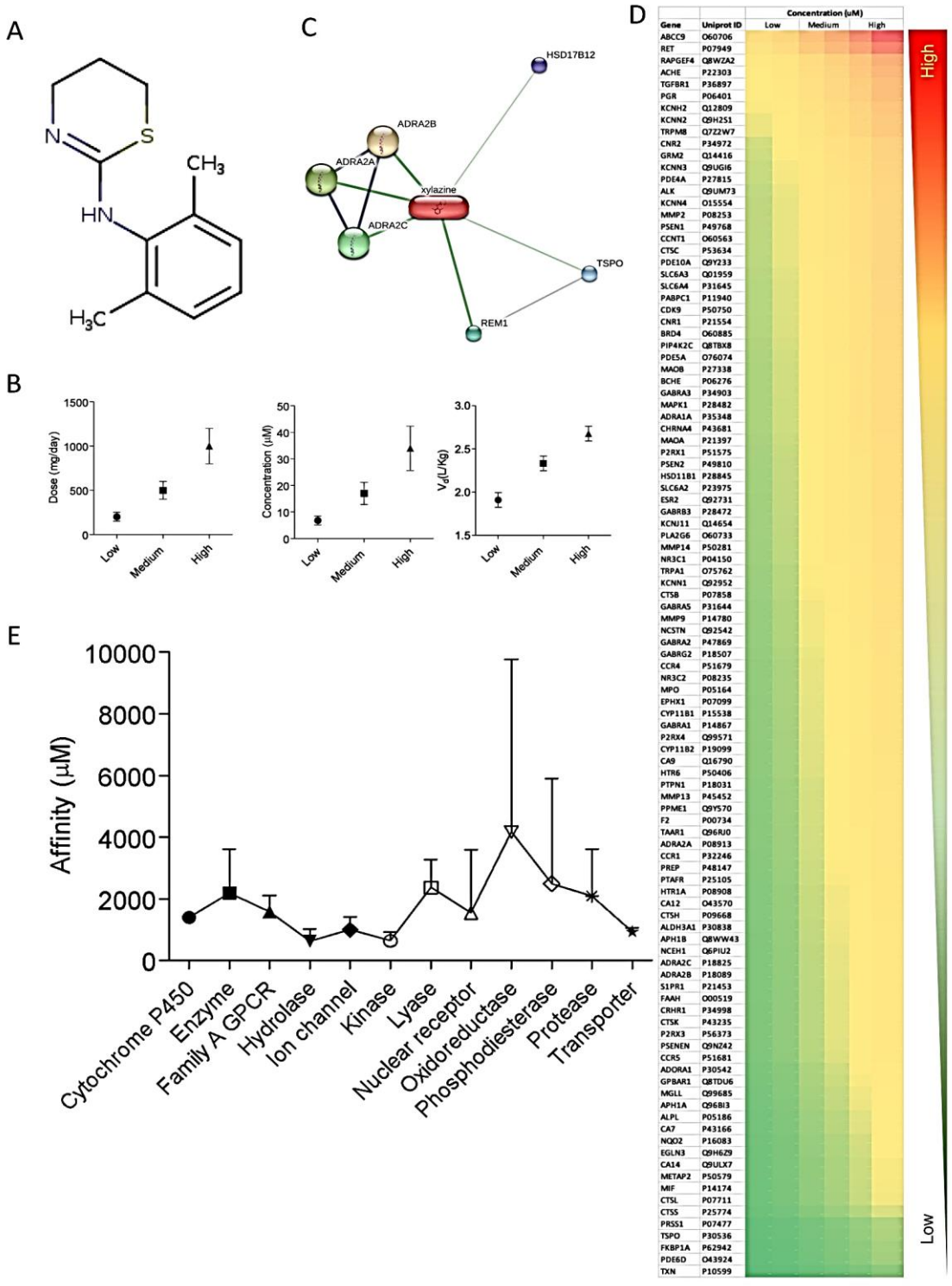
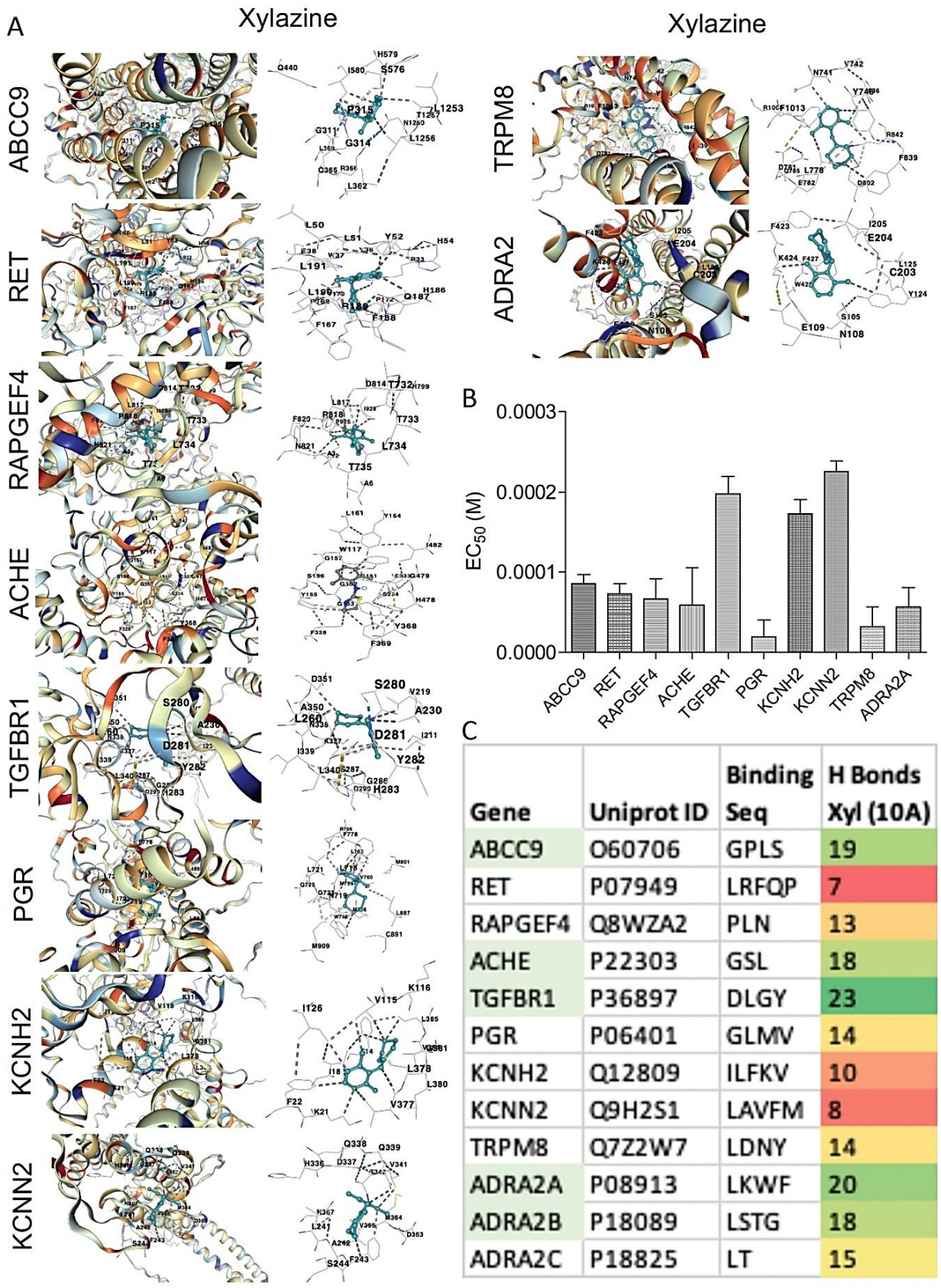


Figure 1: Pharmacological properties of xylazine. The chemical structure of xylazine (A), along with its network proteins (C) identified in the STITCH database. B) The graphs show the trichotomized (low, medium, and high) data of xylazine dose (mg/day), predicted plasma concentration (μM), and volume of distribution (L/Kg) in humans. D) The heat map represents the concentration affinity ratio of xylazine against all its identified targets from the Swiss Target Prediction database (Scale: red to green = high to low ratio values). E) The graph represents the affinity (μM, mean±SD) of xylazine against all its target categories identified from the Swiss Target Prediction database.



Gene	Uniprot ID	Binding Seq	H Bonds Xyl (10A)
ABCC9	O60706	GPLS	19
RET	P07949	LRFQP	7
RAPGEF4	Q8WZA2	PLN	13
ACHE	P22303	GSL	18
TGFBR1	P36897	DLGY	23
PGR	P06401	GLMV	14
KCNH2	Q12809	ILFKV	10
KCNN2	Q9H2S1	LAVFM	8
TRPM8	Q7Z2W7	LDNY	14
ADRA2A	P08913	LKWF	20
ADRA2B	P18089	LSTG	18
ADRA2C	P18825	LT	15

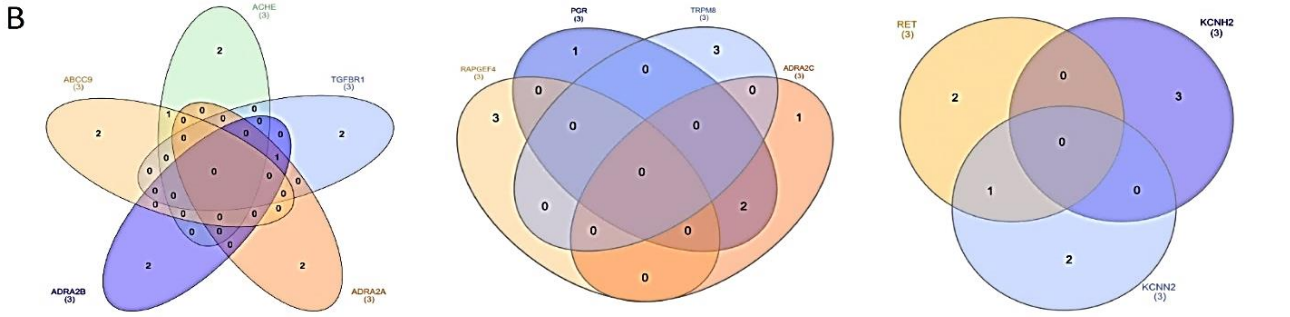
Figure 2: Xylazine and its high-affinity targets. A) Representative images from molecular docking showing the interaction of xylazine with its high-affinity targets. B) The bar graph represents the EC_{50} values (molar (M), mean ± SD) of xylazine for its high-affinity targets. C) Table shows the amino acid sequence of the binding pockets of the xylazine's high-affinity targets along with the number of hydrogen bonds formed at 10Å distance (the targets forming ≥ 18 hydrogen bonds are highlighted in light green).

cerebellum, cholangiocytes, heart, hypothalamus, monocytes, Langerhans cells, liver, Paneth cells, skeletal muscle, and smooth muscle (Figure 3). Of these xylazine targets, ABCC9, ACHE, TGFBR1, and ADRA2C were predominantly expressed on skeletal and smooth muscles (Figure 3 A, B). RET, RAPGEF4, and KCNN2 were considerably expressed in the adrenal gland (Figure 3 A, B), suggesting that the major adverse effects of xylazine could be consequences to compromised physiology of the adrenal gland and skeletal and smooth muscles. To identify the drug that can optimally antagonize xylazine pharmacology, all its high-affinity targets were screened for affinity against selected alpha2AR-antagonists/modulators and CNS stimulants that are clinically used (Figure 3C). A ratio of the affinities of xylazine and selected antagonist to the specific target was estimated to assess the relative potential of the drug to reverse xylazine pharmacology (Figure 3C). In this screening, prazosin was identified as the most optimal drug to reverse xylazine pharmacology with ~ 200 to 2000 (average 660) fold superior affinity against all high-affinity targets of xylazine. This was followed by spiperone (26-fold), yohimbine (21-fold), phentolamine (14-fold), and alprenolol (13-fold) (Figure 3C).

A

Gene	Uniprot ID		Major Expression		Function
ABCC9	O60706	Sk muscle	Liver	Heart	Activation of K-ATP channels, sleep regulation
RET	P07949	Parathyroid	Mid brain	Adrenal gland	Kinase, several functions, including feed intake
RAPGEF4	Q8WZA2	Cortex	Adrenal gland	Cerebellum	Exocytosis
ACHE	P22303	Sk muscle	Hypothallamus	Cerebellum	Enzyme
TGFBR1	P36897	Placenta	Sm muscle	Bone marrow	Kinase, prolifiration and fibrosis
PGR	P06401	Endometrium	Sm muscle	Cervix	Steroid hormone signalling
KCNH2	Q12809	Pituitary	Bone marrow	Cerebellum	K channel (V gated)
KCNN2	Q9H2S1	Liver	Adrenal gland	Hippocampus	K channel (V independent), synaptic hyperpolarization
TRPM8	Q7Z2W7	Prostate	Liver	Testis	Na, K and Ca channels
ADRA2A	P08913	Paneth cells	Sm muscle	Colangiocytes	Adrenergic signalling
ADRA2B	P18089	ionocytes	Langerhans cells	Sm muscle	Adrenergic signalling
ADRA2C	P18825	Endometrium	Sm muscle	Secretory cells	Adrenergic signalling

B



High (Green) Medium (Purple) Low (Orange)

C

		Affinity (uM)														
Gene	Uniprot ID	Xylazine	Yohimbine	Chlorpromazine	Phentolamine	Mianserine	Spiperone	Prazosin	Alprenolol	Propanolol	Pindolol	Atipamezole	Dexmedetomidine	Tolazoline	4-Aminopyridine	Doxapram	Caffeine
ABCC9	O60706	147.7															
RET	P07949	185.4															
RAPGEF4	Q8WZA2	308.7															
ACHE	P22303	351.0															
TGFBR1	P36897	401.9															
PGR	P06401	404.6															
KCNH2	Q12809	471.0															
KCNN2	Q9H2S1	516.0															
TRPM8	Q7Z2W7	519.4															
ADRA2A	P08913	1590.6															
ADRA2B	P18089	1912.8															
ADRA2C	P18825	1891.3															

Figure 3: Expression and antagonism of high-affinity targets of xylazine. A) The major expression of (top three tissues) high-affinity targets of xylazine are shown along with their major function. B) The Venn diagrams trichotomize (High: Green, medium: purple, and low: orange) the expression pattern of xylazine targets based on their affinities. C) The heat map represents the affinity ratios of xylazine and its antagonist to the high-affinity targets of xylazine (scale: green represents the lowest ratio indicating better antagonism, while red represents the highest ratio indicating poor antagonism).

Discussion

The results of this study contribute significantly to the understanding of xylazine's pharmacological profile and have provided insights into optimally reversing its effects in humans. The comprehensive investigation into the pharmacological profile of xylazine has unveiled details of its dose-dependent pharmacokinetics, molecular interactions, and potential adverse effects and identified promising reversal agent/s. This study expands beyond the pharmacological effects of xylazine mediated by activation of alpha-2 adrenergic receptors and offers know-how on the possibilities of xylazine interacting with other targets with much superior affinity in humans. There are two major reasons to justify the study observations: i) In human patients with XUDs, the clinical symptoms observed are not classical of alpha-2 adrenergic receptor activation (Ayub et al., 2023; Sibbesen et al., 2023) and ii) The clinical approach to correcting adverse effects in XUD patients using alpha-2 adrenergic receptor antagonist show very poor efficacy (Ball et al., 2022; Quijano et al., 2023; Sibbesen et al., 2023). These two reasons justify that the adverse effects observed in XUDs are mediated possibly by receptors other than alpha-2 adrenergic receptors, and this study specifically identifies nine targets (ABCC9, RET, RAPGEF4, ACHE, TGFBR1, PGR, KCNH2, KCNN2, and TRPM8) which can be involved in XUDs. These specific targets of XUD identified offer a platform to develop effective and targeted reversal agents.

The delineation of xylazine doses into low, medium, and high categories, along with the associated estimation of plasma concentrations in humans, has significant implications for comprehending the reversal of xylazine pharmacology. Recognizing how different doses impact plasma concentrations is fundamental for devising effective reversal protocols, specifically in the context of concentration affinity ratios of the drugs. The plasma concentration ranges defined in this study are consistent with previous reports (Ball et al., 2022; Quijano et al., 2023; Sibbesen et al., 2023) and offers valuable insights into the pharmacokinetics/dynamics of xylazine and its reversal in the human body. This information becomes pivotal for clinicians seeking to design protocols that efficiently and safely counteract the adverse effects of xylazine. Categorizing xylazine doses also allows for an assessment of the potential risks associated with reversal at higher doses, where plasma concentrations are elevated to influence many more targets. This understanding is crucial for balancing the efficacy of reversal agents with safety considerations for the patient (Ehrman-Dupre et al., 2022; Kacinko et al., 2022; Malaca et al., 2023). This knowledge also aids in optimizing the choice and dosage of reversal agents to achieve prompt and effective reversal while minimizing adverse effects. This study by characterization of xylazine doses and their corresponding plasma concentrations, considering variable volumes of distribution, significantly enhances the understanding of reversing xylazine pharmacology in humans. This information serves as a cornerstone for developing targeted and safe reversal protocols, ensuring effective mitigation of xylazine-induced adverse effects while prioritizing patient well-being. The observed ranges in this study also offer a foundation for future research on the pharmacokinetics of xylazine in diverse patient populations.

The potential targets of xylazine identified in this study highlight its pharmacological profile, which is crucial to assessing the major adverse effects of xylazine reported in human XUD patients (Mulders et al., 2016; Ayub et al., 2023; D'Orazio et al., 2023; Zagorski et al., 2023) While the pharmacological effects of xylazine through the adrenergic receptors are well known, this study identifies several significantly higher affinity targets of xylazine, which are previously not reported. The highest average affinities of xylazine to target categories such as hydrolases, kinases, transporters, and ion channels suggest a broad impact on several cellular processes potentially regulated by ABCC9, RET, RAPGEF4, ACHE, TGFBR1, PGR, KCNH2, KCNN2, and TRPM8. Of these, the least EC_{50} of xylazine was observed for PGR, followed by TRPM8, ADRA2A, ABCC9, RET, RAPGEF4, and ACHE. The interplay between xylazine and alpha-2 adrenergic receptors reinforces the established role of these receptors in mediating sedation and analgesia (Hovda et al., 2011; Ruiz-Colón et al., 2014; Bates, 2015; Ball et al., 2022). The involvement of TRPM8, ADRA2A, ABCC9, RET, RAPGEF4, and ACHE as xylazine targets expands our understanding of potential signaling cascades beyond direct adrenergic receptor interactions (Hovda et al., 2011; Bates, 2015; Ball

et al., 2022; Kacinko et al., 2022), This interconnected network underscores the need for a holistic approach when studying the pharmacodynamics of xylazine. Identification of high-affinity targets such as TRPM8, ADRA2A, ABCC9, RET, RAPGEF4, and ACHE aligns with recent reports on adverse effects associated with xylazine abuse, which are not classical of alpha-2 adrenergic receptor stimulation (Ruiz-Colón et al., 2016; Ehrman-Dupre et al., 2022; Ayub et al., 2023; Pergolizzi Jr et al., 2023). The tissue-specific expression of these targets, especially in the liver, adrenal gland and skeletal/smooth muscles, is also consistent with clinical observations of xylazine's impact on microvasculature, endocrine and musculoskeletal systems (Ruiz-Colón et al., 2014; Mulders et al., 2022; Ayub et al., 2023; Pergolizzi Jr et al., 2023; Sibbesen et al., 2023; Zagorski et al., 2023). This correlation supports the notion that the undesired effects of xylazine can be attributed to its interactions with the specific targets identified in this study. The observation of xylazine interaction with KCNN2 and KCNH2 is consistent with most clinical reports of adverse cardiovascular effects in XUD patients (Quijano et al., 2023; Sibbesen et al., 2023). Xylazine was also observed to target ACHE, which can lead to enhanced acetylcholine levels, together with its sympatholytic effects (Ball et al., 2022; Malaca et al., 2023), can severely imbalance autonomic physiology, leading to compromised cardiovascular and respiratory functions, which are consistently observed in XUD patients (Ball et al., 2022; Malaca et al., 2023; Sibbesen et al., 2023; Zagorski et al., 2023). XUD patients also suffer from altered sleep patterns (Ball et al., 2022; Malaca et al., 2023; Sibbesen et al., 2023), and in this study, the identification of ABCC9 as a target of xylazine provides mechanistic insight into these pharmacological effects observed. ABCC9, which is a subunit of ATP-sensitive potassium channels, is known to regulate sleep duration (Allebrandt et al., 2013; Parsons et al., 2013; Nelson et al., 2015; Yücel et al., 2020) possibly by influencing the diffuse modulatory system (Allebrandt et al., 2013; Köles et al., 2016) through compromised functions of RET and RAPGEF4,(Zhou et al., 2016; Brüning et al., 2019; Nguyen et al., 2019) which are also high-affinity targets of xylazine. In addition to the effects of xylazine on autonomic, endocrine, and musculoskeletal systems, it is likely to influence sensory physiology as well by targeting TRPM8, which is a receptor-activated non-selective cation channel involved in the detection of cold (<25°C) and intracellular pH (Andersson et al., 2004; Fujita et al., 2013). Such broad-spectrum pharmacology of xylazine poses several challenges when treating its adverse effects.

The involvement of multiple targets of xylazine in its observed adverse effects necessitates optimally targeting them for effectively reversing its effects. Identification of such an approach is only possible through network pharmacology analysis (Singh et al., 2023; Kumar, 2024). The results of the screening for high-affinity targets of xylazine and their antagonism by selected alpha-2 adrenergic receptor-antagonists and CNS stimulants provide valuable insights into potential candidates for reversing xylazine pharmacology. In this study, prazosin emerged as the most optimal drug for reversing xylazine pharmacology, demonstrating a remarkable 200 to 2000-fold superior affinity against all high-affinity targets of xylazine. Hence, prazosin could be a highly effective antagonist for mitigating the effects of xylazine, making it a promising candidate for further investigation and potential clinical use. The identification of prazosin as a potent antagonist aligns with previous research on alpha-2 adrenergic receptor antagonists, emphasizing their potential clinical applications (Cavero and Roach, 1980; Yücel et al., 2020), and this study further expands on the clinical utility of prazosin through its effects independent of alpha-2 adrenergic receptors. The currently used treatment options for xylazine toxicity include various combinations of intravenous fluids, saline ocular irrigation, mechanical ventilation, saline cathartics, gastric lavage, silver sulfadiazine cream/antibacterial ointments and yohimbine/tolazoline/atipamezole/naloxone (1.2 to 2 mg), lidocaine/atropine/magnesium infusion /activated charcoal/metoprolol succinate/ thiamine /clonidine, all of which have shown only limited clinical efficacy in XUD patients (Ball et al., 2022; Ayub et al., 2023; Malaca et al., 2023; Quijano et al., 2023; Sibbesen et al., 2023) Considering the current clinical limitations, the potential use of prazosin in reversing xylazine pharmacology suggested in this study offers a detailed mechanistic insight which merits its clinical evaluation. In comparison with other antagonists evaluated in this study, prazosin

stands out as a particularly potent antagonist, and hence, evaluating its efficacy in XUD patients should be considered to validate prazosin's effectiveness in real-world scenarios and explore its safety profile in diverse patient populations.

Conclusion

In summary, this study provides a robust mechanistic understanding of the pharmacological properties of xylazine, offering insights into potential adverse effects and avenues for targeted interventions. These insights into xylazine use-associated adverse effects could be relevant to all species. The identified high-affinity targets, network interactions, and the promising role of prazosin as a reversal agent set the stage for future investigations in XUD patients and animals.

Article Information

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Conflict of Interest. The authors declare no conflict of interest.

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