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Effects of Xylazin on sedative, analgesic and some liver enzymes in rats

Efectos de la Xilazina sobre los efectos sedantes, analgésicos y algunas enzimas hepáticas en ratas

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ABSTRACT

This study was conducted to determine the sedation level of rats after using two different doses of Xylazine, to measure the damage that may occur in the liver, and to determine the change in pain response due to sedation. The use of analgesic substances is one of preferred methods in pain control studies. Analgesic effects are also present in drugs used for sedative purposes. Xylazine is used for pain relief, calming (sedative), sleep inducing (hypnotic), and striated muscle relaxation. It is also used for premedication purposes before using different anesthetic drugs. 24 rats used in the study were randomly selected and divided into three groups. Only physiological saline was given to the rats in the control group (KO). The second group was given Xylazine (K10) 10 mg/kg intraperitoneally (ip), and the third group was given Xylazine (K15) 15 mg/kg/ip. Sedation assessment was performed according to the behavior of the rats to which sedative drugs were applied to the stimuli. Sedation was classified as mild, moderate and severe. The degree of pain-relieving properties of Xylazine in rats was measured with a pain test. The pain test was performed with a hotplate test and a hotplate apparatus. After the pain tests, the rats were sacrificed after taking blood as needed. AST, ALT and corticosteroid values were measured in the blood serum. In this study, it was determined that Xylazine had pain-relieving properties and did not change liver enzyme values when used at a dose of 15 mg/kg in rats, and it was concluded that it could be preferred for safe sedation in cases where short-term complete immobility was needed.

Key words: Pain; rat; sedation degree; Xylazine

RESUMEN

Este estudio se realizó para determinar el nivel de sedación de ratas después de usar dos dosis diferentes de Xilacina, y medir el daño que puede ocurrir en el hígado y para determinar el cambio en la respuesta al dolor debido a la sedación. El uso de sustancias analgésicas es uno de los métodos preferidos en los estudios de control del dolor. Los efectos analgésicos también están presentes en los medicamentos utilizados con fines sedantes. La Xilacina se utiliza para aliviar el dolor, calmar (sedante), inducir el sueño (hipnótico) y relajar el músculo estriado. También se utiliza con fines de premedicación antes de usar diferentes medicamentos anestésicos. Se seleccionaron al azar 24 ratas utilizadas en el estudio y se dividieron en tres grupos. Solo se administró solución salina fisiológica a las ratas del grupo de control (KO). El segundo grupo recibió xilacina (K10) 10 mg/kg por vía intraperitoneal (ip), y el tercer grupo recibió xilacina (K15) 15 mg/kg/ip. La evaluación de la sedación se realizó de acuerdo con el comportamiento de las ratas a las que se les aplicaron fármacos sedantes ante los estímulos. La sedación se clasificó como leve, moderada y severa. El grado de propiedades analgésicas de la xilacina en ratas se midió con una prueba de dolor. La prueba de dolor se realizó con una prueba de placa calefactora y un aparato de placa calefactora. Después de las pruebas de dolor, las ratas fueron sacrificadas después de extraerles sangre según fuera necesario. Se midieron los valores de AST, ALT y corticosteroides en el suero sanguíneo. En este estudio, se determinó que la xilacina tuvo propiedades analgésicas y no modificó los valores de las enzimas hepáticas cuando se usó una dosis de 15 mg/kg en ratas, y se concluyó que podría ser la opción preferida para una sedación segura en casos en los que se necesitara inmovilidad completa a corto plazo.

Palabras clave: Dolor; rata; grado de sedación; Xilacina

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INTRODUCTION

Sedation is a common procedure used to reduce the response of an animal that needs to remain calm to external stimuli, to facilitate small applications and to increase safety [1]. It is the depression of the central nervous system with drowsiness due to the application of sedative drugs. It is the calming of the animal by putting the central nervous system under mild pressure. The patient is relaxed and calm despite being awake [2].

Such drugs are used in patients to reduce fear and anxiety, to minimize the total dose of anesthetic to be administered, to provide easier induction, to provide easier and more comfortable recovery from anesthesia, to provide pain relief, to reduce bronchial and saliva secretion, and to eliminate the vasovagal reflex [3]. The sedative and analgesic effects depend on the activation of the supraspinal and spinal regions of alpha2-adrenoceptors [4 , 5]. The primary purpose of using alpha2-adrenoceptor agonists is to cause sedation in animals. In addition to these effects, they also cause analgesia due to their central and spinal effects [6].

In mammals, alpha2-adrenoceptors are found in many tissues and systems such as the gastrointestinal tract, uterus, and platelets, and their stimulation causes different effects. Sedation and analgesia occur as a result of stimulation of alpha 2-adrenoceptors located in the central nervous system [6]. Their effects on the respiratory system vary according to animal species. In dogs (*Canis familiaris*), cats (*Felis catus*) and horses (*Equus caballus*), doses that provide advanced sedation may reduce the respiratory rate and, to a lesser extent, decrease the Partial Arterial Carbon Dioxide Pressure ($PaCO_2$) value. In ruminants, tachypnea may occur, respiration is provided with effort and the $PaCO_2$ level decreases [6].

Requirements for ideal sedation

First of all, the animals must be hungry. A calm environment/ room is required before and after the injection for the maximum effect of sedative drugs. There should be no animal sounds of the same species/breed and different genders. Especially for cats and caged poultry, there should be no sounds of animals of the dog type. For pets, there should be no general noise or buzzing in the environment, as well as the smell of previous animals, and if possible, there should be dim lighting [2 , 6].

Pain

The most valid definition of the concept of pain today was made by the International Association for the Study of Pain (IASP). According to this organization, pain; It is an unpleasant sensory and emotional experience that accompanies existing or potential tissue damage or can be defined by this damage [7 , 8].

Animal pain studies definitely require animal material. The more suitable this material is for the study, the more accurate the results are. The assessment of pain in animals is quite problematic. An animal that cannot express itself verbally will show itself by giving similar reactions in similar events. Its response is usually simple reflexes, sometimes in the form of vocalization or escape. What is important in such experiments is to learn when the animal perceives pain. Methods using mechanical, thermal and chemical stimuli have been developed to understand the presence of pain [9 , 10].

Xylazine

Xylazine is a non-narcotic compound used in veterinary medicine as a sedative, analgesic, and muscle relaxant, and is often referred to as “anestesia de caballo” or “horse anesthesia”. It is not approved for human use as an antihypertensive because of its extreme depression of the central nervous system, profound hypotension, but is introduced for veterinary use as a sedative, emetic, analgesic, and muscle relaxant [11]. It is a premedicant drug used before anesthesia in the vast majority of animal species. It is applied by iv. or im. in horses *(Equus caballus)*, cattle (*Bos taurus*), sheep (*Ovis aries*), goats (*Capra hircus*), dogs *(Canis familiaris),* cats *(Felis catus),* pigs (*Sus scrofa domesticus*) and poultry (*Gallus gallus domesticus*). It produces varying degrees of muscle relaxation and analgesia depending on the animal species [6 , 12]. Xylazine is often used together with ketamine as an anesthetic agent, especially in experimental studies on rabbits *(Leporidae)* and rats *(Rattus)* [11].

After Xylazine application, cardiac output may decrease by 1/3 or even 1/2 and blood pressure may decrease by 1/4 to 1/3. These effects occur in almost all species. After the application of the recommended clinical dose of Xylazine, respiratory rate decreases, while arterial pH, $PaCO_2$ and PaO_2 levels do not change in cats and dogs, while there is a minimal decrease in horses [6].

Xylazine and sedation degrees

Some researchers classified the stages after the onset of sedation in sheep with Xylazine into 3 as mild, moderate and severe [13]. Mild sedation occurs when Xylazine is administered to sheep at 0.1 mg/kg/iv [14]. In cattle, mild, moderate and severe sedation has also been classified [2 , 6]. In a study investigating the clinical effects of Xylazine premedication on water buffalo calves (Bubalus bubalis) castrated under general anesthesia, 10 animals were administered 0.1 mg/kg/im and 0.15 mg/kg/im Xylazine, anesthesia was induced with Ketamine (2 mg/kg/iv) and maintained with Isoflurane-Oxygen-air. Safe and consistent sedation was achieved in both groups, which were evaluated every 5 min [15]. Topal [6] reported that administering 1 mg/kg/ iv Xylazine to horses caused mild sedation. In a study in which 1.1 mg/kg/iv. xylazine was administered to seven clinically healthy male adult miniature donkeys *(Equus asinus)* and the degree of sedation was controlled, it was determined that mild or moderate sedation was observed in the donkeys between 5 and 60 min after treatment and that no sedation was observed after 90 min, and that all animals recovered from sedation without complications within 2 h [16]. In another study conducted in Sri Lanka on 15 young Asian elephants *(Elephas maximus)*, the first signs of sedation occurred within 5-18 min after injection of Xylazine (50-110 mg per elephant; 0.09-0.15 mg/kg/im), and all elephants were sedated in standing position. Twelve elephants remained standing during the sedation period, while 3 elephants were reported to have recumbent [17].

Pain classification according to origin

According to the region where it originates, pain is classified as visceral, somatic, sympathetic and peripheral pain. Visceral pain can be widespread, difficult to localize and reflected. It is also generally called pain originating from internal organs. Somatic pain is a type of pain that originates from somatic nerves, starts suddenly, is sharp and well localized. Vascular pain that occurs with the activation of the sympathetic nervous system is called sympathetic pain. The source of peripheral pain can be muscle, tendon or peripheral nerves [18].

Pain classification according to mechanisms

a-Nociceptive pain: Free nerve endings that respond to thermal, mechanical and chemical stimuli that cause tissue damage are called "nociceptors". The entire series of events that create the pain felt with tissue damage is called "nociception" [19]. The processes that occur in physiopathology occur as a result of the stimulation of pain receptors called nociceptors. Nociceptors are found in all tissues and organs, as well as in the nervous system [20].

Nociceptive analgesia is perceived in four physiological stages [21 , 22]. In the periphery; transduction and transmission, in the medulla spinalis; modulation, in the transmission to the central nervous system; perception plays a role [23].

b-Neuropathic (non-nociceptive) pain: The International Association for the Study of Pain (IASP) reports neuropathic analgesia as a pain caused by damage or dysfunction of any part of the nervous system [24]. The formation of neuropathic pain occurs as a result of compression, cut, ischemia, infiltration or damage of neurons. The source of neuropathic pain occurring in any part of the body may be the peripheral nervous system, central nervous system or autonomic nervous system. Accordingly, it is classified as centrally or peripherally sourced neuropathic pain [25].

Evaluation of neuropathic pain models in animals

Von frey test

Von frey filaments are of various thicknesses (0.25-2 g) and are performed to detect tactile allodynia. In order to detect allodynia, measurements should first be made on a normal subject. In the von frey test, different filaments are pressed against the subject's skin in the mid-plantar areas for measurement. A total of five presses are made at a frequency of twice per second. If the animal withdraws its paw, this filament is recorded as the threshold value in the records. Over time, a decrease in the threshold will be observed in applications made to the extremity. In order to talk about tactile allodynia, it must withdraw its paw significantly with the lowest stimulus [9].

The subject is placed in a closed test box and the device is placed so that it coincides with the subject. With the automatic version, the values of the mechanical pain threshold are observed with the Dynamic Plantar Anesthesiometer. With the help of a mirror, a force that can increase up to 50 g is applied to the plantar surface of the animal's hind paw and as the animal withdraws its paw, the value on the device's screen is accepted as the nociceptive threshold value. This value is automatically recorded by the device [9].

Hotplate test

The hotplate test is performed by placing a mouse (*Mus musculus*) or rat (*Rattus norvegicus*) in a gap with an open-ended cylinder placed on a metal surface. Although first described by Woolfe and MacDonald in 1944. It usually consists of a surface (copper or aluminum) heated to 50-56 degrees. In order for the animal to remain on the heated surface in certain areas, it must be used in glass cylinders that are large enough to not restrict its movements. The time elapsed from the moment it is placed on the surface until the animal withdraws its hind leg is observed. It is one of the most preferred methods in the sources. On the other hand, the observation criteria for the animal feeling the sensation of pain vary. There are problems in understanding which of the behaviors it shows is a pain indicator. After a constant temperature is achieved, the reaction time of a mouse or rat placed in a cavity is used to evaluate two behaviors. The behavior shown may be pulling the animal's foot, or it may be licking, kicking, shaking, dancing or jumping. When examining the animal's licking behavior, the licking of the hind legs is taken into consideration more. Because it can lick its front legs at normal times. The test is terminated when the animal jumps because it tends to try to escape when it touches the ground. The time after the test is terminated is recorded. The average duration of the reactions a mouse can give varies between 5-20 s. In order not to cause tissue damage, the test should not be applied for more than 30 s [9 , 10].

This study was conducted to determine whether Xylazine has analgesic properties after injection of a sedative dose into rats, to reveal the damage it may cause to the liver, and to clinically grade sedation in rats.

MATERIALS AND METHODS

Twenty four female, adult and healthy Wistar albino rats were used in the study. Rats were housed in an environment with an average temperature of 20-24 °C and 50-60% humidity, with 12 h of light and 12 h of darkness. Standard pellet rat feed and drinking water were given *ad libitum* for feeding the rats.

Rats were randomly divided into three groups, with 8 rats in each group. Hotplate device (May AHP 0603 Analgesic Hot Plate, Commat, 2018, Turkey) (FIG. 1) and Modified hotplate apparatus (10 cm handle, 15 mm diameter and 20 mm height brass cylinder) were used for the tests. The apparatus was applied for testing when it reached 56 °C (Sous vide cooker, Profi Cook PC-SV-1126, 2019, France) (FIG. 2A-B).

Control group (KO group n=8): Each animal in this group was injected with 0.10 mL of Physiological Saline (NaCl 0.9%) intraperitoneally.

Xylazine 10 mg group (K10 group, n=8): In order to create sedation, 10 mg/kg dose of Xylazine was injected intraperitoneally to the animals in this group.

Xylazine 15 mg group (K15 group, n=8): In order to create sedation, 15 mg/kg dose of Xylazine was injected intraperitoneally to the animals in this group.

FIGURE 1. Hotplate device used in the study

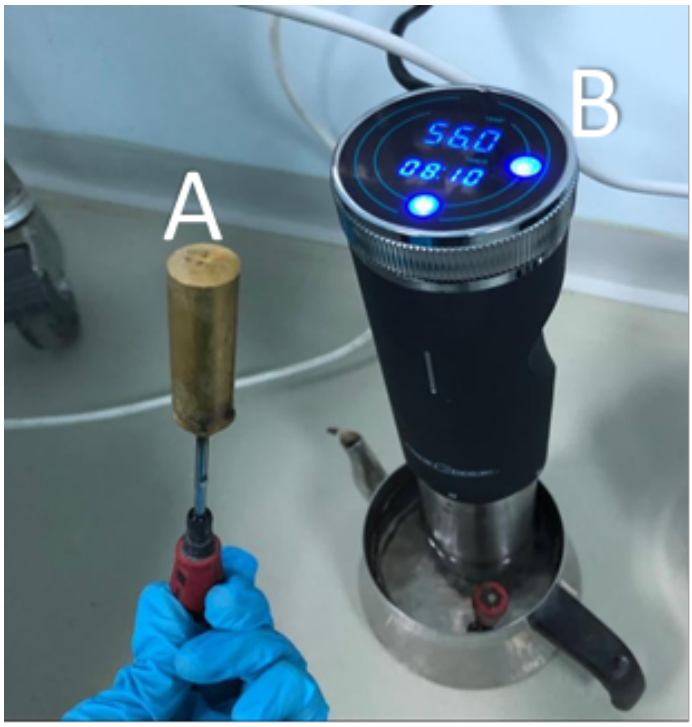

FIGURE 2. Hotplate apparatus (A) and temperature stabilizer (B)

Sedation assessment

After administering physiological saline in the KO group and sedative drugs in the K10 and K15 groups, observations were made at the 1st, 2nd, 3rd, 4th, 5th, 10th, 15th, 20th, 30th and 45th min to grade sedation. To evaluate sedation, rats were placed in transparent 15-liter containers immediately after injection and monitored without being disturbed. When they became immobile during observation, they were stimulated audibly and mechanically by hitting two metal pieces together (a 6-8 cm metal plate was hit 2-3 times). Their reactions to attempts to catch, hold, and turn were checked. The disappearance of the Righting Reflex (RR) (coming to lateral or dorsal recumbency) and the time for RR return (the animal coming to sternal recumbency after lying lateral or dorsal) were checked. After the sedative agent was applied, the movements and behaviors of the animals were monitored and sedation was graded according to the criteria below, using the sedation classifications of many researchers. The presence and evaluation of sedation were done separately for each rat [13 , 26 , 27].

No sedation (Degree 0): The animal is active, mobile, and has the activation to respond to all kinds of environmental stimuli. It makes an escape movement during the manual capture process with the help of a towel or cloth. It can bite and scratch when caught. RR is present.

Mild Sedation (Degree 1): Its mobility has decreased but it is still active. It may be sleepy, it may wait in lateral recumbency. It may stand or walk slowly. It may lean/hit the edges of the container or cage wires it is in. Its reactions have weakened after the manual capture process. RR has not disappeared.

Moderate Sedation (Degree 2): It is very sleepy, there is dorsal or lateral recumbency. However, it may walk slowly or make different foot/head movements during the manual capture process with the help of a towel or cloth. RR is still present.

Severe Sedation (Degree 3): It is immobile and unresponsive to environmental stimuli in lateral, abdominal or dorsal recumbency. It remains in the same position when its position is changed. There is no response during and after the capture/holding process. In the lying position, movements such as tail, leg and partial tremors in the head can be observed. RR is always negative.

Conducting a temperature test to evaluate the analgesic effect

To determine whether Xylazine has pain-relieving properties in rats, two materials with the same temperature were used. In the pre-sedation period, rats that were not sedated were placed on a hot surface in the sternal (abdominal) position in the hotplate device and tested. After sedation began, a pain test was performed using the hotplate apparatus. When a rat is placed on a hot surface in the hotplate device heated to 56 °C, it will be able to change its body position, perform a flexor reflex or lick its foot due to the pain/ache it experiences. However, when it is placed on a hot surface in the same device in the sternal position during the sedation period, it will not be possible for it to change its body position or perform a flexor reflex even if it feels the pain or ache. Therefore, since misleading results may occur, it was found appropriate to develop a hotplate apparatus and check the presence of flexor or extensor reflexes against heat at the same temperature in the lateral lying position under sedation.

In order to keep a rat that has not been sedated in the lateral position before sedation, it is necessary to hold it tightly from the nape and tail base to reduce its movements. In addition, the rat will make an intense effort to get out of this position. It was thought that these procedures would affect the state/time of response to the hot apparatus applied to the sole of the foot and that incorrect results would occur. Considering these two situations, the pain test was performed with a hotplate device before sedation and with a hotplate apparatus with the same temperature during sedation.

Acclimation to the device before sedation

In order for the rats to get used to the hotplate device environment before sedation, each rat was kept on the device until it came out of the glass globe or for 3 min while the device was closed and then taken from the device and placed in their cages.

Performance of the pain test with the hotplate device before sedation (BS)

After the device was turned on before the sedative drug was applied and reached a constant temperature of 56°C, each of the 24 rats was placed on the hot surface and their behaviors were evaluated in terms of determining the reaction time of the rat. Each rat was monitored for behavioral changes by two observers. After the rat was placed on the hot floor, it was evaluated whether it quickly pulled/lifted its legs away from the hot floor (flexor reflex), licked the sole surfaces of its feet or jumped. Lifting and licking the forelimbs from the floor is a behavior that can occur at normal times. However, it was also taken into account that the time of lifting and licking the feet from the floor may be different due to the hot floor.

Performing the pain test with the hotplate apparatus during sedation (DS).

One liter of normal water was placed in a suitable container and started to be heated with a circulatory water heater (FIG. 2B). When the water temperature reached 56 °C, this process was automatically terminated by the device and the water temperature was kept constant due to its feature. The modified hotplate apparatus was kept inside from the time the water started to heat up.

Ten min was waited for the mild sedation that occurred after the sedative agent was administered. At the end of this period, the pain test was performed considering that the rat would not pass to the moderate/severe level of sedation. Pain reflex test was performed at the 10th min in rats with moderate sedation and at the 10th min after severe sedation. When the test was to be performed under sedation, the rat was first placed in the right and then left lateral recumbency position and the upper forefoot and hindfoot soles were touched by holding the handle of a modified hotplate apparatus at 56 °C (FIGS. 6-7). The time between the rat's withdrawal of its foot (flexor reflex, withdraw reflex) after the foot sole was touched was recorded. Serum aspartate transaminase (AST), Alanine aminotransferase (ALT) and cortisol parameters were measured (DAS Elisa Reader, 2019, Halomedicals Systems Limited, Italy) to determine the liver damage status.

Variance analysis (ANOVA), Duncan multiple comparison and Paired Samples T test for dependent samples were applied in the statistical evaluation of the data (SPSS 22).

RESULTS AND DISCUSSION

Clinical results in the KO group

No changes were detected in the behavior of the rats in the control group during the 45 min after the physiological saline injection.

Clinical results in the K10 group

As seen in TABLE I, it was observed that 7 out of 8 rats entered sedation at the 5th min, 6 of them entered the moderate sedation stage at the 10th min and remained in this state until the time of awakening, and 2 of them entered the severe sedation stage very quickly from mild sedation (TABLE I).

TABLE I. Sedation levels determined in the K10 group

K10 Group	1. min	2. min	3. min	4. min	5. min	10. min	15. min	20. min	30. min	45. min
K10-1	0	0	0	0	1	1	2	2	1	—
K10-2	0	0	0	0	1	1	3	3	2	—
K10-3	0	0	0	0	1	3	3	3	2	—
K10-4	0	0	0	0	2	2	2	2	1	—
K10-5	0	0	0	0	1	2	2	2	1	—
K10-6	0	0	0	1	1	2	2	2	1	—
K10-7	0	0	0	0	0	2	2	2	1	—
K10-8	0	0	0	1	1	2	2	2	1	—

0: no sedation, 1: mild sedation, 2: moderate sedation, 3: severe sedation, min: minute, —: no evaluation was made.

At the 30th min, it was observed that the current sedation state in all rats had eased and at the 45th min, it was thought that there was no need to evaluate sedation and the monitoring process was terminated. Blood samples were taken from all rats and the rats were sacrificed. The blood samples were centrifuged and the serum was separated.

Clinical results in the K15 group

As seen in TABLE II, all rats were sedated at the 5th min, 7 of them were in moderate sedation and 1 was in severe sedation at the 10th min. Sedation continued regularly and without any problems until the 20th min except for 1. A total of 3 rats were in severe sedation (TABLE II) (FIGS. 3, 4 and 5).

TABLE II. Sedation levels and sedation durations in the K15 group.

K15 Group	1. min	2. min	3. min	4. min	5. min	10. min	15. min	20. min	30. min	45. min
K15-1	0	0	0	0	1	2	2	3	3	2
K15-2	0	0	0	0	1	2	3	3	2	—
K15-3	0	0	0	1	2	2	2	2	1	—
K15-4	0	0	0	1	1	2	2	2	1	—
K15-5	0	0	0	0	1	3	2	2	1	—
K15-6	0	0	0	0	1	2	2	2	1	—
K15-7	0	0	0	1	1	2	2	2	1	—
K15-8	0	0	1	1	1	2	2	1	—	—

0: no sedation, 1: mild sedation, 2: moderate sedation, 3: severe sedation, min: minute, —: no evaluation.

In the 30th min control, it was observed that sedation had eased in 7 of the rats. In the 45th min control, it was observed that the degree of sedation had eased in the last one, and the monitoring process was terminated. Blood samples were taken from all rats and the rats were sacrificed. The blood samples were centrifuged and their serums were separated.

Pain test results

It was determined that the rats placed in the hotplate device for the purpose of accustoming them first sniffed the device, calmly walked around inside it, and then tried to get out. It was observed that some of them sat on their haunches and calmly licked their front feet and some their hind feet.

After being placed in the heated device to perform the pain test before sedation, the sniffing and walking process was performed again. However, it was determined that seconds later, they quickly pulled their feet off the ground (flexor reflex), then pulled their other foot, quickly licked it, then pulled their other foot and quickly licked it, and quickly got out of the container. When these behaviors were determined, the rat was taken off the device. It was determined that the foot pulling and licking behavior was much faster than normal behaviors.

A pain test was performed with the hotplate device before applying physiological saline to the rats in the KO group. However, since no sedation occurred as a result of the physiological saline application, pain testing could not be performed with the hotplate apparatus. When the hotplate apparatus at 56 °C was applied to the feet of the rats under sedation (K10, K15), only flexor reflexes occurred in the subjects. The mean reflex times in the KO, K10 and K15 groups are presented in TABLE III.

TABLE III. The mean pain test reflex times of all groups.

Groups	BS (second)	DS (second)	P
KO	11.62	—	
K10	12.37	13.50	0,051
K15	13.12	17.87	0,017*

—: test could not be performed. * Since p<0.05, there is a statistically significant difference between BS and DS in the K15 group. BS: Mean duration of foot withdrawal, licking and jumping behaviors on the hotplate device before sedation. DS: Mean duration of foot withdrawal behavior after contact with the hotplate device during sedation

In TABLE III, the pain sensitivity and response times of rats in the K0, K10 and K15 groups before and during sedation were measured and their average times were compared. Accordingly, it was observed that the reflex times were longer in the K10 and K15 groups both before and during sedation than in the KO group. In addition, the fact that the response times of the K15 group were significantly longer than in the K10 group before and during sedation showed that better sedation was provided.

FIGURE 3. Rat in lateral recumbency during the sedation phase.

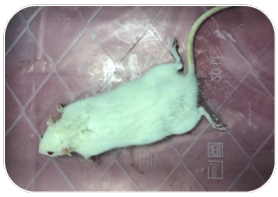

FIGURE 4. Sternal recumbency position of the rat under sedation.

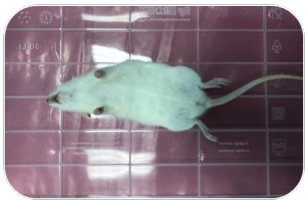

FIGURE 5. Dorsal recumbency position of the rat under sedation.

FIGURE 6. Performing the pain test with the hotplate apparatus under sedation.

FIGURE 7. Performing pain test with hotplate apparatus under sedation.

Biochemical results

Serum AST, ALT and cortisol values of all groups are presented in TABLE IV.

TABLE IV. Mean values of biochemical parameters

Groups	KO	K10	K15	Mean	P
AST	53.5000	52.1250	72.8750	59.5000	0.089
ALT	27.2500	25.3750	30.3750	27.6667	0.546
Cortisol	1.6138[b]	2.2025[a]	2.2763[a]	2.0308	0.001*

* Since p<0.05, there is a significant difference between the groups (ANOVA – analysis of variance).
Duncan multiple comparison test was used. There is a statistically significant difference between those with different letters (a, b, c)

In TABLE IV, serums of all rats were taken and liver enzymes (AST, ALT) and cortisol values were measured and the average values of these biochemical parameters were compared. Accordingly, while no difference was observed in terms of AST and ALT enzymes in the K0, K10 and K15 groups, it was observed that the cortisol value was lower in the K0 group and was close to each other in the K10 and K15 groups and there was no significant difference.

Xylazine was first reported in animals in the late 1960s and is currently approved for use in veterinary medicine as a nonopioid tranquilizer. Xylazine is frequently used in conjunction with ketamine as an anesthetic in experimental studies in dogs, cats, horses, rabbits, and rats [11]. Xylazine has been implicated in significant morbidity and mortality in the United States and worldwide in recent years due to its multidrug use and its different mechanism of action compared to illicit opioids such as heroin and fentanyl. Therefore, it is extremely important to be aware of the threat that these powerful drugs pose when used illicitly [28]. Karasu and Gençcelep [13] classified the stages after the start of sedation in sheep with Xylazine into 3 as mild, moderate and severe. Carvalho [14] stated that mild sedation occurs when Xylazine 0.1 mg/kg/iv is administered to sheep. Mild, moderate and severe sedation classifications have also been made in cattle [2 , 6]. Mild sedation occurs when Xylazine 1 mg/kg/iv is administered to horses [6]. It has been reported that mild, moderate and severe sedation occurs in cats and dogs using Xylazine and other chemical agents [12 , 29 , 30]. In a study evaluating the effectiveness of Xylazine-Ketamine anesthesia on reflexes and vital signs during and after tendon surgery in rabbits; Xylazine (5 mg/kg/im) and Ketamine (35 mg/kg/im) injections were administered to rabbits, and reflexes, ear pinch reflex and pedal reflex were measured before and after the anesthesia injection. It was stated that when Xylazine-Ketamine was administered together, the return of reflexes was delayed compared to the group administered only Ketamine, the duration of surgical anesthesia was longer, and adequate anesthesia was provided for the rabbits as evidenced by good cardiovascular and other clinical indices [31]. In the study, it was determined that three different degrees of sedation emerged in both groups by looking at the movement/behavioral changes of rats that were administered Xylazine at two different doses. It was also observed that the quality of sedation was better in the K15 group than in the K10 group.

Karasu and Gençcelep [13] determined the lateral recumbency of the animal as the limit in the clinical distinction between moderate and severe sedation in their study on sheep. In this study, the loss of the righting reflex in rats was evaluated as the most important distinguishing point of severe sedation.

Xylazine is frequently applied to horses and ruminants for sedation and analgesia. The sleepiness after Xylazine application may continue for 1-2 h, but the analgesic effect is as short as 15-30 min. Painful interventions should be performed within 10-15 min of the sedative effect. It is known that Xylazine is more effective than opioids in eliminating pain in horses and ponies [6]. Xylazine is an alpha-2 adrenergic receptor stimulant that was first used in veterinary medicine for analgesic and sedative purposes [32]. It is reported that Xylazine has analgesic properties according to animal species. Gençcelep and Karasu [27] stated that in the sedation they performed with Xylazine in sheep, it did not have analgesic properties in mild and moderate sedation, but mild analgesic properties in severe sedation.

In the study, the fact that the response times after contact with the hot plate apparatus in the K10 and K15 groups were longer than in the KO group was evaluated as the presence of the analgesic property of Xylazine in rats. However, the fact that the serum cortisol level had different values compared to the control group was interpreted as the analgesic property of Xylazine being very low.

Cortisol is a major glucocorticoid that is necessary for life and is found after physiological stress. Cortisol levels remain high for a long time after trauma and systemic stress [18]. Some researchers reported that Xylazine reduces serum cortisol levels, but cortisol levels increase under the influence of different stresses [33].

In a study conducted on ten healthy female Merino meat sheep; sedation with Xylazine was applied to sheep lying on their backs, assuming that hoof trimming creates a short but intense stress situation, and there was no increase in respiratory rate and rectal temperature, but a decrease in heart rate, and a decrease in escape, defense and general stress behavior (reduction in head and leg movements, sitting on the knees and licking) were observed. Serum cortisol concentrations were also measured at 2.28 times lower levels compared to control sheep [34].

In the study, the fact that the response times were longer in the K10 and K15 groups after the contact with the hot plate apparatus compared to the KO group was evaluated as the presence of the analgesic property of Xylazine in rats. The increase in serum cortisol levels in the K10 and K15 groups compared to the KO group was thought to be due to the pain stress resulting from the contact of the hot plate apparatus with the animals' feet.AST and ALT biochemical values are used to evaluate the damage status in the liver in terms of laboratory. In the liver, muscle damages and metabolic disorders, the levels of Aspartate amino transferase (AST) and Alanine amino transferase (ALT) enzymes in the blood are important in terms of indicators of damage [35 , 36 , 37].

Enzymes produced in hepatocytes by liver cells are the first structures that determine the damage in the liver. Under normal conditions, liver cells store these enzymes. However, when an injury or damage occurs in the liver cells, these enzymes mix with the blood and can be detected with blood tests. The enzymes that are specific to the liver and are most commonly used to reveal liver damage are amino transferases (AST and ALT). AST and ALT tests are among the tests used in the diagnosis of hepatocellular damage [38]. As a result of this study, the fact that no change was detected in serum AST and ALT values was interpreted as Xylazine did not cause any damage to the liver.

In a study on castration of water buffalo calves, it was emphasized that among the two groups premedicated with 0.1 mg/kg/im and 0.15 mg/kg/im Xylazine, a faster and more satisfactory sedation occurred in the group administered 0.15 mg/kg/im Xylazine [15].

CONCLUSION

In this study, especially in rats, which are frequently used as experimental animals for many diseases, treatments, medications, surgical interventions and other experimental purposes; It was determined that Xylazine could be administered at doses of 10 and 15 mg/kg/ip as a sedative, analgesic and muscle relaxant, and could be used easily and safely as it did not change AST and ALT values and therefore did not cause liver damage. In addition, it was concluded that when 15 mg/kg/ip Xylazine is used, its pain-reducing properties are significantly increased and it can be preferred for safe sedation in cases where short-term complete immobility is needed.

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Conflict of interest

The authors declare that there are no conflicts of interest.

BIBLIOGRAPHIC REFERENCES

[1] Wagner MC, Hecker KG ,Pang DSJ. Sedation levels in dogs: a validation study. BMC Vet. Res. [Internet]. 2017[cited December 24 2024]; 13:110. Available in: https://goo.su/5HKUTeQ

[2] Ünsaldı S. Veteriner Anestezi. Birinci Baskı. İstanbul: Nobel Tıp Kitapevleri; 2011. p. 28-66.

[3] Rioja E, Santos M, Martínez-Taboada F, Albancovichi JA, Tendillo FJ. Cardiorespiratory and minimum alveolar concentration sparing effects of a continuous intravenous infusion of dexmedetomidine in halothane or isoflurane-anaesthetized rats. Lab. Anim. [Internet]. 2006; 40(1):9-15. doi: https://doi.org/fcn8v5

[4] Buerkle H, Yaksh TL. Pharmacological evidence for different alpha 2-adrenergic receptor sites mediating analgesia and sedation in the rat. Br. J. Anaesth. [Internet]. 1998; 81(2):208-215. doi: https://doi.org/pfw8

[5] Guo TZ, Jiang JY, Buttermann AE, Maze M. Dexmedetomidine injection into the locus ceruleus produces antinociception. Anesthesiology. [Internet]. 1996; 84(4):873-881. doi: https://doi.org/cw4ftz

[6] Topal A. Veteriner Anestezi. Birinci Baskı. Bursa: Nobel Tıp Kitapevleri; 2005. p. 41-48.

[7] Raj PP. Ağrı taksonomisi. In: Erdine S, editor. Ağrı. Birinci baskı. İstanbul: Alemdar Ofset; 2000.

[8] Merskey H, Bogduk N. Descriptions of chronic pain syndromes and definition of pain terms, Seattle: IASP Press; 1994

[9] Kartal F, Özyalçın NS. Hayvanlarda akut ve kronik ağrı modellerinin değerlendirilmesi. In: Önal A, editor. Algoloji. Birinci Baskı. İstanbul: Nobel Tıp Kitabevi; 2004. p. 299-324.

[10] Bennett GJ. Animal models and their clinical implications. In: Merskey H, Loeser JD, Dubner R, (ed). The Paths of Pain 1975-2005. Seattle: IASP Press; 2005. p. 243-251

[11] Papudesi BN, Malayala SV, Regina AC. Xylazine Toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025[cited Jul. 17 2024]. Available in: https://goo.su/WBuPM1M

[12] Koç B, Sarıtaş K. Veteriner Anesteziyoloji ve Reanimasyon. Ankara: Medipres yayınevi; 2004. p. 80-94.

[13] Karasu A, Gençcelep M. The Effect of Xylazine HCl Used in Repeated Sedations for Sheep on Biochemical and Clinical Values. Kafkas Univ Vet Fak Derg. [Internet]. 2015; 21(6):831-836. doi: https://doi.org/pfxc

[14] Carvalho LL, Nishimura LT, Borges LP, Cerejo SA, Villela IO, Auckburally A, de Mattos- Junior E. Sedative and cardiopulmonary effects of xylazine alone or in combination with methadone, morphine or tramadol in sheep. Vet. Anaesth. Analg. [Internet]. 2016; 43(2):179-188. doi: https://doi.org/f8j6fp

[15] Guerri G, Cerasoli I, Straticò P, De Amicis I, Giangaspero B, Varasano V, Paolini A, Carluccio A, Petrizzi L. The Clinical Effect of Xylazine Premedication in Water Buffalo Calves (Bubalus bubalis) Undergoing Castration under General Anaesthesia. Animals. [Internet]. 2021; 11(12):3433. doi: https://doi.org/pfxd

[16] Samimi A. Evaluation of the sedative and clinical effects of xylazine, detomidine, medetomidine and dexmedetomidine in miniature donkeys. NZVJ. [Internet]. 2019; 68(3):198–202. doi: https://doi.org/pfxg

[17] Jansson T, Perera BV, Edner A, Fahlman A. Standing sedation with xylazine and reversal with yohimbine in juvenile asian elephants (*Elephas maximus*). J. Zoo. Wild. Med. [Internet]. 2021; 52(2):437-444. doi: https://doi.org/gkkcwm

[18] Morgan GE, Mikhail MG . Pain Management. In: Clinical Anesthesiology, 2nd ed. New Jersey: Prenttice-Hall Interntional, Inc; 1996. p. 274-316.

[19] Cousins M, Power I. Acute and postoperative pain. In: Wall PD, Melzack R, editors. Textbook of Pain. 4th ed. London. Churchill Livingstone; 1999. p. 447-491.

[20] Gürel ÖK. Değişik Muskuloskeletal Sistem Hastalıklarının Basınç Ağrı Eşiği Üzerine Etkisi [specialization thesis on the Internet]. İstanbul: Haydarpaşa Numune Eğitim ve Araştırma Hastanesi Fiziksel Tıp Ve Rehabilitasyon Kliniği; 2009. [Accessed December 23 2024]. Available in: https://goo.su/dFDq

[21] Keskinbora K, Aydınlı I. An atypical opioid analgesic: Tramadol. Pain. [Internet]. 2006 [Accessed 23 December 2024]; 18(1):5-19. Available in: https://goo.su/za7Kua

[22] Grond S, Sablotzki A. Clinical pharmacology of tramadol. Clin. Pharmacokinet. [Internet]. 2004; 43(13):879-923. doi: https://doi.org/dq5s8c

[23] Uçkunkaya N, Gurbet A. Baş ve yüz ağrıları. In: Güldoğuş F, Güleç S, Uçkunkaya N, editörler. Ağrı fizyolojisi. Birinci Baskı. Ankara: Güneş Kitabevi; 2007. p. 1-11.

[24] Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T, Serra J. Neuropathic pain: redefinition and a grading system for clinical and research purposes. Neurology. [Internet]. 2008; 70(18):1630–1635. doi: https://doi.org/bgm4bw

[25] Köknel Talu G. Nöropatik Ağrı. In: Erdine S, editor. Ağrı. Üçüncü Baskı. İstanbul: Nobel Tıp Kitabevi; 2008. p. 461-471.

[26] Jang HS, Lee MG. Effects of medetomidine on analgesia and sedation in rats. J Vet Clin. [Internet]. 2010[Accessed December 23 2024]; 27(6):674-678. Available in: https://goo.su/2t2ex0

[27] Gençcelep M, Karasu A. Evaluation of analgesic and sedative effects of repeated and increasing doses of xylazine HCl in sheep. Med. Weter. [Internet]. 2017; 73(8):468-472. doi: https://doi.org/pfxk

[28] Edinoff A, Sall S, Upshaw W, Spillers N, Vincik LA, Witt A, Murnane K, Kaye Adam and Kaye Alan. Xylazine: A Drug Adulterant of Clinical Concern. Current Pain and Headache Reports. [Internet]. 2024; 28(5): 417-426. doi: https://doi.org/pfxm

[29] Gomes VH, Monterio ER, Dias RS, de Olivera RLS, Alburquerque-da Silva MF, Coelho K. Comparison of the sedative effects of morphine, meperidine or fentanyl, in combination with acepromazine, in dogs. Cienc. Rural. [Internet]. 2011; 41(8):1411-1416. doi: https://doi.org/c5598q

[30] Bell AM, Auckburally A, Pawson P, Scott EM, Flaherty D. Two doses of dexmedetomidine in combination with buprenorphine for premedication in dogs; a comparison with acepromazine and buprenorphine. Vet. Anaesth. Analg. [Internet]. 2011; 38(1):15-23. doi: https://doi.org/d527nq

[31] Hassan MA, Abd-Elkawi M, Saber MS, Senosy W, Nassif M, ElSherif MW. Evaluation of Xylazine Ketamine Anesthesia in Rabbits Undergoing Tendon Surgery: A Prospective Randomized Controlled Study. NVVJ. [Internet]. 2024; 4(1):1-7. doi: https://doi.org/pfxs

[32] Bilgili A, Altıntaş L, Şahindokuyucu F. The use of Sedative and Immobilizan Drugs in Cats and Dogs. YYU Vet Fak Derg. [Internet]. 2003 [Accessed December 22 2024]; 14(1):77-82. Available in: https://goo.su/cTzCiUy

[33] Fayed AH, Zakaria AD, Hedaya SA, El-Ashmawy IM. Effect xylazine (alpha 2-adrenergic agonist) on the stress response to immobilization and heat in rats. AJAS. [Internet]. 1994[Accessed December 23 2024]; 7(3):397-400. Available in: https://goo.su/g6A5dS

[34] Amin S, Wagner R, Fieseler H, Rizk A, Schmicke M, Spilke J, Mielenz N, Baumgartner W, Schären-Bannert M and Starke A. Sedation with xylazine hydrochloride decreases the stress response in merino meat sheep during routine hoof trimming in a tilt table. Front. Anim. Sci. [Internet]. 2022; 2:795543. doi: https://doi.org/pfxx

[35] Mert N. Veteriner Klinik Biyokimya. 1th. ed.Baskı. İstanbul: Ceylan Matbaacılık;1997. p. 230-240.

[36] Dolar E. Klinik Karaciğer Hastalıkları. 1th. ed.Baskı. Ankara: Nobel&Güneş Tıp Kitabevi; 2002. p. 133-146.

[37] Guyton AC, Hall JE. Tıbbi Fizyoloji. 12th. ed. Baskı. İstanbul: Nobel Tıp Kitapevi; 2013. p. 937- 942.

[38] Ersoy O. Evaluation of elevated liver enzymes. Ankara Med J. [Internet]. 2012 [Accessed December 22 2024]; 12(3):129-135. Available in: https://goo.su/wBoq2ya