# The sedative effect of intranasal administration of some sedative agents in budgerigar *(Melopsittacus undulatus*)

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## Abstract

This study was assigned to evaluate the sedative effect of intranasal administration of xylazine or diazepam (with or without Chitosan) in budgerigar. A pilot study conducted to determine the effective dose of each drug that cause adequate sedation in these birds and on which the experiment 2 was built. It involved slowly administration of various increasing doses with equal volumes of each drug into each nostril. The dose va1ued 26 and 12 mg/kg. BW. for xylazine and diazepam respectively. In experiment 2, the onset of action, duration of sedation, and duration of dorsal recumbency induced by administratIon of each drug at its effective dose were studied. The combined intranasal administration of chitosan with xylazine or diazepam on sedation quality also was investigated. Intranasal administration of xylazine or diazepam at their effective dose caused adequate sedation in budgerigar where xylazine caused significant prolongation ($P<0.05$) in duration of sedation than that induced by diazepam, although xylazine failed to induce dorsal recumbency as that induced by diazepam. A combined administration of chitosan with xylazine or diazepam caused significant prolongation ($P<0.05$) in both duration of sedation induced by xylazine and duration of sedation and dorsal recumbency induced by diazepam compared to each agent when given alone indicating an obvious absorption enhancer effect of chitosan The best results were obtained after administration of diazepam- chitosan formulation. It was concluded that intranasal administration of xylazine or diazepam produce rapid and adequate sedation, also chitosan enhance the absorption of both xylazine or diazepam when administered in conjunction.

# التأثير المسدر لبعض العوامل المسدرة في طيور الحب *Melopsittacus undulates* عن طريق الأنف

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## الخلاصة

صُممت هذه الدراسة لتقييم الفعل المسدر الناتج عن إعطاء الزيلازين أو الدايزيبام عن طريق الأنف بشكل منفرد أو مع الكايتوسان في طيور الحب. شملت الدراسة أجراء تجربتين، تضمنت التجربة الأولى دراسة أولية لتحديد الجرع المناسبة لكلا الدوائين، واللازمة لأحداث تسدير ملائم في هذه الطيور، والتي بنيت على أساسها التجربة الثانية حيث تضمنت الإعطاء البطيء عن طريق الأنف بجرع متزايدة وبحجوم ثابتة من كلا الدوائين، بلغت هذه الجرع 26 و12 ملغم/ كغم من وزن الجسم لكل من الزيلازين والدايزيبام على التوالي. أما في التجربة الثانية فقد تم دراسة الوقت اللازم لأحداث التسدير، مدة التسدير، ومدة الرقاد الظهري المحدثة بواسطة أعطاء الجرعة المناسبة لكل دواء. كما وتم دراسة تأثير الإعطاء عن طريق الأنف لمزيج الكايتوسان والزيلازين أو الكايتوسان والدايزيبام على نوعية التسدير، أدى الإعطاء الأنفي للزيلازين أو الدايزيبام وبجرعتيهما المناسبة إلى أحداث تسدير

ملائم في طيور الحب، حيث سبب الزيلازين إطالة معنوية ($P<0.05$) في فترة التسدير مقارنة بما أحدثه الدايزيبام على الرغم من فشل الزيلازين في إحداث الرقاد الظهري الذي أُحدث بواسطة الدايزيبام. إن أعطاء الكايتوسان مع الزيلازين أو الدايزيبام سبب إطالة معنوية ($P<0.05$) في كلاً من مدة التسدير المحدثة بالزيلازين ومدتي التسدير والرقاد الظهري المحدثة بالدايزيبام مقارنة مع كل دواء إذا ما أعطي بشكل منفرد مشيراً إلى وجود تأثير واضح للكايتوسان في تحسين الامتصاص، إن أحسن النتائج التي تم التوصل إليها كانت بعد الإعطاء عن طريق الأنف لمزيج الدايزيبام والكايتوسان. تم الاستنتاج بأن الإعطاء عن طريق الأنف للزيلازين أو الدايزيبام تسبب وخلال فترة قصيرة في أحداث تسدير ملائم، كما وإن الكايتوسان حسن وبشكل واضح امتصاص كلاً من الزيلازين أو الدايزيبام عند مزجه مع أي منها.

## Introduction

Many procedures in caged birds such as wound dressing, blood collection, fracture splinting and some diagnostic procedures require a chemical restraint (sedation or anaesthesia) in order to overcome a lot of associated problems as stress, anxiety and struggling (1) Administration of drugs by inhalation is limited by lack of sophisticated specialized equipments or experience of the administrator (2). So administration of drugs by injection has gained importance as it can overcome these limitations (3). The anatomical characteristics of small pet birds make the injection in these species hazardous, intravenous injection is difficult and intramuscular injection is usually associated with risk, where the pectoral muscle of these birds as finches, canaries and budgerigar have small mass and the intravascular or intracoelomic drug injection can occur in association with injection into this muscle leads to immediate shock and death (4) as well as administration of drugs into the hind limb musculature is not recommended since they may be eliminated too quickly by the kidneys, where birds have active renal portal system that can shift the flow of blood from lower extremities (5) and also intramuscular injection into the thigh most probably associated with nerve damage . Therefore, a technique of drug administration, particularly sedatives, is a challenge in small birds. Intranasal administration provides a useful way of taking a range of systemic drugs, where the drug delivered to the nose may have local and systemic effect (6). This method of administration is convenient and more recently favorable rates of absorption and metabolism have been demonstrated (7). It was discovered empirically that several drugs such as lignocain (8), propranalol (9), progesterone (10), enkephalins (11) and nicardipine (12) were readily absorbed by the nasal mucosa with bioavailabilities similar to intravenous absorption. In order to increase the bioavailability of intranasal route certain agents were used to enhance the absorption of various drugs across the nasal mucosa. Unfortunately some of these, such as bile salts and Laureth- 9 are toxic to the nasal mucosa (6). A polymer obtained from deacetylation of chitin, a naturally occuring structural polymer abundant in carb and shrimp shells known as chitosan [2- amino- 2- deoxy- (1-4)- $\beta-$ D-glucopyranan] which is not toxic to nasal mucosa (6, 13 and 14). It is a mucopolysaccaride soluble in organic acid (acetic acid) or inorganic acid (hydrochloric acid) and positively charged (13). Chitosan has many biological, chemical and physical properties and can be used in a wide range of application in medicine, veterinary practice, cosmetic, food industry, agriculture and biotechnology (15, 16, and 17). Because of its biodegradability and biocompability, chitosan has been applied as a pharmaceutical excipient in oral, ocular, nasal implant and transdermal drug delivery (18). Recent studies indicated that chitosan could enhance absorption of poorly absorbable drugs such as peptides and proteins (19). The aims of this study were: 1) Investigate whether intranasal administration of

sedatives is an effective approach to induce sedation in budgerigar, and then determine the effective dose of xylazine, $\alpha 2$ - agonist, and diazepam, benzodiazepine drug, that has the ability to induce satisfactory sedation in these birds. 2) Examine whether a nasal formulation containing chitosan could exhibit an enhancement effect on the absorption of xylazine or diazepam.

## Materials and Methods

- **Experimental birds:** Thirty healthy adult budgerigars *(Melopsittacus undulatus)* of both sexes were purchased from a local market in AL-Diwaniya city. Their body weight ranged from 18 to 28 gram with an average of 22±0.58 gram. They were fed with seeds and vegetables and had free access to tap water. They were examined to adjudge the health status before the beginning of trial, each bird was used two times at weekly intervals.
- **Drug Preparation:**
- **Preparation of xylazine stock solution:** To 10 ml of distilled water a few drops of hydrochloric acid were added until the pH of this solution became about 4. The solution was added to 50 ml of the patenet xylazine Preperation (Rompun, 20 mg/ml, Bayer, Leverkusen, Germany) to obtain stock solution contains 1.66% xylazine.
- **Preperation of xylazine- chitosan formulation (X-C formiution):** Three grams of chitosan was dispersed in distilled water and hydrochloric acid was added into the above systems, to enhance solubility, under agitation until chitosan was dissolved, to obtain 50 ml of 6% chitosan solution with pH about 4. 10 ml of the previous solution was added to 50 ml of the patent xylazine preperation 2% in order to obtain final solution contains 1.66% xylazine and 1% chitosan (X-C formulation).
- **Preparation of Diazepam stock solution:** To 5 ml of distilled water a few drops of hydrochloric acid were added until the pH of this solution became about 4. The solution was added to 25 ml of the patent diazepam preparation (Valuim, 5 mg/ml, Roche, Madrid, Spain) to obtain stock solution contains 0.41% diazepam.
- **Preparation of diazepam-chitosan formulation (D-C formulation):** Five ml of 6% chitosan solution previously prepared was added to 25 ml of the patent diazepam prepration 0.5% in order to obtain final solution contains 0.41% diazepam and 1% chitosan (D-C formulation).
- **Study 1: Determination the appropriate dose of xylazine or diazepam that induces sedation in budgerigar:** Budgerigars were randomly assigned to groups with 6 birds for each group. Birds in these different groups received equal volumes of different concentrations, prepared from stock solution of xylazine, administered slowly into each nares using a micropipette (Dragon- med, China) in an increasing dosage manner until the effective dose that induced satisfactory sedation was reached i.e birds lost their ability to fly, move or struggle and did not need for further physical restraint when placed in separate cages for observation after drug administration. By the same way mentioned above, the determination of diazepam dose was done after intranasal administration of equal volumes of different concentrations prepared from stock solution of diazepam. It is worth while that all birds were manually fixed in dorsal position during and for 1 minute after the intranasal administration in study 1 and 2.
- **Study 2: Evaluation of the sedative effect of xylazine or diazepam and the absorption enhancer activity of chitosan in budgerigar:** Birds were divided into four groups (A, B, C , D and E), each group with 6 birds. The amount and volume of dose that proved effective in study 1 would be used in study 2,

- Group (A): Administered intranasally with 1% solution of chitosan prepared by dilution of 6% chitosan solution previously prepared.
- Group (B): Administered intranasally with the 1.66% xylazine.
- Group (C): Administered intranasally with X-C formulation (1.66% xylazine and 1% chitosan).
- Group (D): Administered intranasally with 0.41% diazepam.
- Group (E): Administered intranasally with D-C formulation (0.41% diazepam and 1% chitosan).

The following parameters were measured:

- Onset of action.
- Duration of dorsal recumbency.
- Duration of sedation.

## Results and Discussion

In budgerigars, intranasal administration of xylazine at dose of (26 mg/kg. BW) with a volume of( 34 $\mu L$ ),(17 $\mu L$ ) into each nostril, caused adequate sedation within relatively a short time. The treated birds showed signs included immobilization, partially closed eyes, flat wing, retracted neck then birds showed sternal recumbency, but they did not stay in dorsal recumbency. Even higher dose of xylazine (50 mg/kg. BW) failed to induce dorsal recumbency in these birds although the duration of sedation was more prolonged. The onset of action after intranasal administration of diazepam at dose of (12 mg/kg.BW) with a volume of ( 64 $\mu L$ ), (32 $\mu L$ ) into each nostril, was also rapid and the birds became heavily sedated where they did not move when placed in dorsal recumbency. Consequently, the dose of (26 and 12 mg/kg. BW) for xylazine and diazepam respectively were chosen for study 2. The onset of action, duration of dorsal recumbency and duration of sedation are shown in (Table, 1)

**Table (1) Onset of action, duration of sedation and dorsal recumbency after intranasal administration of some sedatives in budgerigars**

| Groups | Onset of action (minutes) | Duration of dorsal recumbency (minutes) | Duration of sedation (minutes) |
|---|---|---|---|
| A | - | - | - |
| B | 1.58±0.30 a | - | 103.50 ± 4.85 a |
| C | 1.42 ± 0.20 a | - | 129.83 ± 7.66 b |
| D | 1.50 ± 0.18 a | 33.50 ± 2.09 a | 62.33 ± 4.11 c |
| E | 1.33 ± 0.25 a | 40.67 ± 2.26 b | 76.17 ± 2.46 d |

**- Figures represent mean + standard error.**
**- Different small letters represent significant differences between groups vertically at (P<0.05).**
**- Similar small letters represent insignificant differences between groups vertically at (P<0.05).**
**- Data were analyzed using one- way analysis of variance.**
**- A: birds treated with chitosan at dose of (29 mg/kg. BW).**
**- B: birds treated with xylazine at dose of (26 mg/kg. BW).**
**- C: birds treated with xylazine at dose of (26 mg/kg. BW). – 1% chitosan formulation.**
**- D: birds treated with diazepam at dose of (12 mg/kg. BW).**
**-E: birds treated with diazepam at dose of (12 mg/kg. BW). – 1% chitosan formulation**

In study 2 the onset of action in all treated groups was rapid , except in group of birds treated intranasally with 1% chitosan alone with a volume of ( 64 $\mu L$ ), (32 $\mu L$ ) into each nostril, where they failed to show sedation, and there were no significant differences between groups indicating that intranasal administration of drugs produce rapid absorption. This was in agreement with previous reports, which showed that the rate of absorption, plasma concentration and pharmacokinetics after intranasal administration often compares well to that obtained by intravenous medication because of the rich vasculature and high permeability of the nasal mucosa where the large number of fenestrated capillaries just below the surface epithelium may well contribute

to the rapid absorption (20). The duration of sedation was significantly ($P<0.05$) longer with xylazine (103.50 ± 4.85) compared with diazepam (62.33 ± 4.11). The duration of dorsal recumbency in diazepam treated group was (33.50 ± 2.093) whereas xylazine did not allow birds to stay in dorsal recumbency (table, 1) that may be due to variation in response to xylazine in Budgerigar compared with other species of animals. The differences in duration of sedation might possibly be attributed to the differences in metabolism of the two drugs. The results of this study indicated that intranasal administration of xylazine or diazepam can provide reliable sedation. The Intranasal sedative and analgesic have been used in human patients both pediatric and adult (21,22). However, intranasal benzodiazipine has been successfully used for sedation and treating seizures as it avoids the discomfort associated with i. v or i.m injection (23, 24). Although the intranasal administration of drugs is not a common procedure in veterinary medicine, xylazine has been used intranasally as effective drug to reduce stress in Elk captured by net gun (25), and diazepam was used intranasally in rabbits and has been shown to provide a rapid absorption and the onset time for the effect was found to be varying within 1.5-4.5 minute (26). This study can be regarded as complementary to and approved by these previous studies. There was remarkable pharmacological effect resulted from incorporation of chitosan with xylazine or diazepam. However, the group treated intranasally with X-C formulation showed significant increase ($P<0.05$) in duration of sedation compared to that administered with xylazine only (table, 1). Also there was a significant prolongation ($P<0.05$) in duration of sedation as well as duration of dorsal recumbency in brids treated with D-C formulation than those treated with diazepam (Table, 1). These results indicated that chitosan at 1% concentration has had a prominent absorptive enhancer activity when incorporated with xylazine or diazepam which might be attributed to two effects of chitosan on nasal mucosa. The mucoadhesive properties of the polymer can reduce the clearance rate of drugs from nasal cavity, thereby prolonging the contact time of chitosan delivery system with nasal epithelium (27). In addition, it has been shown that the interaction of the positively charged amino group of chitosan with the negatively charged sialic acid residues in mucus causes the transient opening between the tight junctions and allows large hydrophilic compounds to be transported across the epithelium. The opening mechanism of the tight junctions has been demonstrated by a decrease in ZO-I protein and change in cytoskeletal proteins F- actin from a filamentous to a globular structure (28 and18). In conclusion, it has been shown that the intranasal instillation of drugs appeared to be an acceptable method for drug delivery in budgerigar and provides a rapid, safe, easy experience and does not required special technical skills, painless and effective non- invasive method of sedation which may supersedes intramuscular or subcutaneous routes in pet birds. Xylazine and diazepam can be used effectively alone or in combination with chitosan to provide adequate sedation, but the best results were obtained after intranasal administration of D-C formulation which provides a reliable sedation in budgerigar. These results indicate that the absorption enhancement activity by chitosan may be of a practical importance in improving intransal drugs delivery system.

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