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# The effect of vitamin C at varying times on physiological parameters in rabbits after xylazine anaesthesia

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**Summary**

The effects of vitamin C administration at varying time intervals on rectal temperature, respiratory rates, heart rates and sleeping time following xylazine anaesthesia was evaluated in rabbits. A total of 36 rabbits placed in six groups (A-F) with 6 animals per group each were used. Groups A and B were used as controls for vitamin C (120 mg/kg, oral) and xylazine (4 mg/kg, intramuscular) treatments, respectively, while groups C-F received vitamin C at four intervals prior to xylazine anaesthesia. The result of the study showed that vitamin C pre-medication prior to xylazine anaesthesia induced depression in respiratory and heart rates and a slight increase in rectal temperature. It also significantly increased sleeping time in rabbits ($p<0.05$). The lengthiest duration of sleep was observed among rabbits that received vitamin C 60 min prior to xylazine anaesthesia. Vitamin C administration 10 min prior to xylazine anaesthesia in rabbits induced a sleeping time three times the value compared to those animals that had received xylazine anaesthesia alone. However, the study did not observe a significant difference ($p>0.05$) in temperature between groups either before or after xylazine administration. It was concluded that vitamin C alters the clinical parameters as well as the sleeping time in rabbits under xylazine anaesthesia.

**Keywords**

Anaesthesia, Physiology, Rabbit, Vitamin C, Xylazine.

## Effetto della somministrazione in tempi variabili della vitamina C sui parametri fisiologici del coniglio dopo anestesia con xilazina

**Riassunto**

*Sono stati valutati nel coniglio gli effetti della somministrazione a intervalli di tempo variabili della vitamina C sulla temperatura rettale (TR), la frequenza respiratoria (FR), la frequenza cardiaca (FC) e il tempo di sonno (TS) dopo anestesia con xilazina. In totale, sono stati utilizzati 36 conigli suddivisi in sei gruppi (A-F) costituiti da 6 animali per gruppo. I gruppi A e B sono stati usati come controllo per i trattamenti con la vitamina C (120 mg/kg, orale) e la xilazina (4 mg/kg, intramuscolare), rispettivamente, mentre i gruppi C-F hanno ricevuto la vitamina C a quattro diversi intervalli di tempo prima dell'anestesia con xilazina. Il risultato dello studio ha mostrato che la premedicazione con vitamina C prima dell'anestesia*

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*con xilazina ha determinato depressione della FR e della FC e un lieve aumento della TR. Inoltre, ha aumentato significativamente il TS nei conigli (p<0,05). La massima durata del sonno è stata osservata tra i conigli che avevano ricevuto la vitamina C, 60 minuti prima dell'anestesia con xilazina. La somministrazione della vitamina C, 10 minuti prima dell'anestesia con xilazina ha indotto un tempo di sonno tre volte superiore rispetto agli animali che avevano ricevuto la sola anestesia con xilazina. Tuttavia, lo studio non ha osservato una differenza significativa (p>0,05) nella temperatura tra i gruppi, prima o dopo la somministrazione della xilazina. Si ritiene che la vitamina C possa alterare i parametri clinici e il tempo di sonno nei conigli sotto anestesia con xilazina.*

**Parole chiave**

Anestesia, Coniglio, Fisiologia, Vitamina C, Xilazina.

## Introduction

Anaesthesia has been defined as the elimination of sensation by the controlled reversible suppression of nervous function (2). It is a state of unconsciousness produced by a process of controlled reversible drug-induced intoxication of the central nervous system in which the patient neither perceives nor recalls noxious stimuli (10). Pre-anaesthetic medication reduces aggression and fear of apprehension, reduces pain and provides pre-emptive analgesia and reduces the amount of anaesthetic needed in order to minimise anaesthetic risk (7, 9).

The most commonly used injectable agents in veterinary medicine are ketamine, diazepam, propofol, xylazine and medetomidine for horses; certain combinations of these agents improve their anaesthetic properties (8, 25). Xylazine hydrochloride possesses a sedative, analgesic, muscle relaxant, immobilising and hypnotic effects in domestic animals (24) and has been used satisfactorily with other drugs for anaesthesia (1, 3, 15, 16, 19), with both smooth and safe recovery periods. It does not present the shortcomings, deficiencies and inconsist-encies experienced with phenothiazine-derived tranquilizers (12). The sedative effect of xylazine appears to be synergistic with a variety of sedatives, analgesics and anaesthetic drugs; such combinations are preferable to overdosing with xylazine to produce the same effects (10).

Pentobarbital (30 mg/kg administered intra-venously) has been advocated in rabbits with a very narrow margin between effective and lethal dose. Combinations of ketamine and xylazine or ketamine and medetomidine have been shown to be more effective in rabbits and rats and are now commonly used with wide margins of safety in rabbits (16). One recent study has demonstrated the potentiatory role of vitamin C on ketamine anaesthesia in rabbits (5). It has been suggested that vitamin C administration prior to ketamine/ xylazine anaesthesia could be used to accelerate the onset of action and increase the duration of anaesthesia, although the exact mechanism by which it does so will require further investigations (11, 15). The effects of stress and anaesthesia could predispose to cardio-respiratory arrests (11), but the administration of vitamin C can reduce both the cost and dose of anaesthetics required for general anaesthesia with minimal risk to the patient's health.

Vitamin C is a water-soluble vitamin found abundantly in citrus fruits, tomatoes, potatoes, leaf vegetables and meat, especially liver (4). Most animals synthesise vitamin C as ascorbate. However, it is also available in synthetic forms, exerting a modulating influence on the central nervous system (17, 18), either physiologically or pharma-cologically (14, 20).

Different methods of inducing anaesthesia have been described in rats. The use of ketamine alone has been reported to cause hypertonus poor muscle relaxation, persistent pain reflex responses and violent recovery from anaesthesia. However, the use of xylazine, an $\alpha$2-agonist compound, in combination with ketamine, produced good anaesthesia in dogs (11, 19). Based on this premise, this study was designed to evaluate the effects of vitamin C pre-medication in rabbits on some physiological parameters following xylazine anaesthesia. It is

 

hypothesised that vitamin C can influence xylazine-induced anaesthesia by altering the physiological parameters via its modulatory effect on central nervous system functions.

## Materials and methods

### Experimental animals

Thirty-six New Zealand white rabbits (12 males and 24 females) weighing 0.85 kg to 1.42 kg and aged between 2 and 3 years were used in this study. These animals were divided randomly into six experimental groups (A-F) that each had 2 males and 4 females. They were housed in wire gauze cages at the Veterinary Pharmacology Laboratory at the University of Maiduguri, Nigeria. They were fed wheat and maize offal as well as spinach *ad libitum*. Fresh water was also given *ad libitum* and the rabbits were allowed to acclimatise for 14 days prior to the commencement of experiment. The experiments were conducted in conformity with standard ethics regarding the care and use of laboratory animals.

### Experimental design

For this experiment, baseline vital parameters including rectal temperature, respiratory rate, and heart rate ,were taken for the six groups of rabbits before the commencement of treatment. A 1% (w/v) aqueous solution of vitamin C (Em-Vit-C®, Emzor Pharmaceutical industries, Lagos) was prepared and administered orally to the rabbits at a dose rate of 120 mg/kg body weight (as described by Egwu *et al.*, unpublished data). Xylazine (Xylazin®, Indian Immunological Limited, Gallapadu) was administered intramuscularly at a rate of 4 mg/kg body weight.

Animals in groups A and B received only vitamin C and xylazine treatments, respectively, while groups C, D, E and F received vitamin C treatments at different intervals of 10, 20, 30 and 60 min, respectively, prior to xylazine anaesthesia. For each of these four groups treated with vitamin C (C-F), the rectal temperature, respiratory and heart rates were measured before vitamin C and after xylazine treatments. The onset and duration of action on sleeping time in each of these groups were observed and measured according to the method described by Flecknell (9). The respiratory and heart rates were measured with the stethoscope, while the rectal temperature was measured with a rectal thermometer.

### Statistical analysis

The statistical differences between the controls and the pre-medicated groups were analysed using the one-way analysis of variance (ANOVA) (21), while the unpaired student's t-test was used to evaluate the differences before and after treatment. Statistical significance was considered at $p$ value below 0.05.

## Results

The results of the effects of vitamin C (120 mg/kg administered orally) administration at various intervals on xylazine anaesthesia are presented in Tables I, II, III and IV. Table I shows that the administration of xylazine alone produced a rise in rectal temperature of 0.53%, while treatment with vitamin C alone triggered a 1.83% rise in rectal temperature. Pre-treatment with vitamin C at 10, 20, 30 and 60 min before xylazine treatment increased rectal temperature by 1.56%, 1.61%, 3.66% and 1.29%, respectively. The mean rectal temperature change before and after xylazine treatment across treatment groups is shown in Figure 1. However, there were no significant differences ($p$>0.05) in rectal temperature for all the groups treated with vitamin C.

In Table II, xylazine treatment alone increased the respiratory rate by 7.31%, while vitamin C treatment alone depressed respiratory rate by 21.52%. The result also showed that vitamin C treatment at 10, 20 and 60 min depressed the respiratory rate by 26.17%, 14.89% and 28.17%, respectively. However, vitamin C treatment at 30 min prior to xylazine anaesthesia resulted in an increased respiratory rate of 5.98%. The mean respiratory rate change before and after xylazine treatment across treatment groups were significant ($p$<0.05) at 10 and 60 min, respectively, but there was no significant difference ($p$>0.05) at 30 min (Fig. 2).

Table I
Effects of vitamin C on mean rectal temperature

| Experimental groups | Mean rectal temperature (°C) ± SD BAX | AAX | Change (%) |
|---|---|---|---|
| Group A | | | |
| Vitamin C | 38.2 ± 0.66 | 38.9 ± 0.32 | +1.83 |
| Group B | | | |
| Xylazine alone | 37.9 ± 0.54 | 38.1 ± 0.67 | + 0.53 |
| Group C | | | |
| Vitamin C and xylazine at 10 min | 38.4 ± 0.78 | 39.0 ± 0.48 | +1.56 |
| Group D | | | |
| Vitamin C and xylazine at 20 min | 37.2 ± 0.12 | 37.8 ± 0.52 | + 1.61 |
| Group E | | | |
| Vitamin C and xylazine at 30 min | 38.2 ± 0.92 | 39.6 ± 1.02 | +3.66 |
| Group F | | | |
| Vitamin C and xylazine at 60 min | 38.8 ± 0.35 | 39.3 ± 0.45 | +1.290 |

SD standard deviation
BAX before administration of xylazine
AAX after administration of xylazine
+ increase compared to value prior to administration

Table II
Effects of vitamin C on mean respiratory rate

| Experimental groups | Mean respiratory rate (breaths/min) mean ± SD BAX | AAX | Change (%) |
|---|---|---|---|
| Group A | | | |
| Vitamin C | 158 ± 13.25 | 124 ± 6.75 | –21.52 |
| Group B | | | |
| Xylazine alone | 123 ± 20.20 | 132 ±40.12 | +7.31 |
| Group C | | | |
| Vitamin C and xylazine at 10 min | 149 ± 25.26 | 110 ±30.65 | –26.17 |
| Group D | | | |
| Vitamin C and xylazine at 20 min | 141 ±35.65 | 120 ±20.35 | -14.89 |
| Group E | | | |
| Vitamin C and xylazine at 30 min | 117 ± 20.21 | 124 ±35.16 | +5.98 |
| Group F | | | |
| Vitamin C and xylazine at 60 min | 142 ± 30.15 | 102 ± 14.78 | –28.17 |

SD standard deviation
BAX before administration of xylazine
AAX after administration of xylazine
+ increase compared to value prior to administration
– decrease compared to value before administration

Table III shows a decrease of 39.58% in heart rate following xylazine treatment alone, while vitamin C treatment alone induced an increase of 55.66% in heart rate. Pre-treatment of rabbits with vitamin C at 10, 20, 30 and 60 min prior to xylazine anaesthesia resulted in a decrease in heart rate of 9.33%, 11.39%, 15.15% and 22.75%, respectively. The mean heart rate change before and after xylazine treatment across treatment groups showed a significant

depression ($p$<0.05) in the groups treated at 30 min and 60 min (Fig. 3).

The effect of vitamin C on sleeping and wake-up time in rabbits treated with xylazine is shown in Table IV. Xylazine treatment alone produced 3.7 min sleeping time. Rabbits that received vitamin C alone did not sleep, while those that were treated with vitamin C at 10, 20, 30 and 60 min prior to xylazine treatment slept for 9.1, 6.6, 10.5 and 21.5 min, respectively. The mean sleeping time change across treatment groups is shown in Figure 4. There was an increase in sleeping time from the groups pre-medicated with vitamin C at 20 min-60 min, which was significant ($p$<0.05) at 60 min.

Table III
Effects of vitamin C on mean heart rate (HR)

| Experimental groups | Mean heart rate (beats/min) ± SD | | Change (%) |
|---|---|---|---|
| | BAX | AAX | |
| Group A | | | |
| Vitamin C | 106 ± 7.85 | 165 ±40.32 | +55.66 |
| Group B | | | |
| Xylazine alone | 144 ± 52.46 | 87 ±17.12 | –39.58 |
| Group C | | | |
| Vitamin C and xylazine at 10 min | 150 ± 12.46 | 136 ±11.7 | –9.33 |
| Group D | | | |
| Vitamin C and xylazine at 20 min | 158 ±40.30 | 140 ±35.40 | –11.39 |
| Group E | | | |
| Vitamin C and xylazine at 30 min | 165 ± 23.42 | 140 ±11.20 | –15.15 |
| Group F | | | |
| Vitamin C and xylazine at 60 min | 167 ± 23.50 | 129 ± 30.50 | –22.75 |

SD standard deviation
BAX before administration of xylazine
AAX after administration of xylazine
\+ increase compared to value prior to administration
– decrease compared to value before administration

Table IV
Effects of vitamin C on mean sleeping time

| Experimental groups | Mean sleeping time of xylazine anaesthesia (min) ± SD | | Change (%) |
|---|---|---|---|
| | Onset of sleep | Wake-up time | |
| Group A | | | |
| Vitamin C | NSO | NSO | NSO |
| Group B | | | |
| Xylazine alone | 6.7 ± 0.98 | 10.4 ±1.15 | 55.22 |
| Group C | | | |
| Vitamin C and xylazine at 10 min | 7.1 ± 0.68 | 16.2±1.04 | 128.12 |
| Group D | | | |
| Vitamin C and xylazine at 20 min | 11.9±0.43 | 18.5±1.12 | 55.46 |
| Group E | | | |
| Vitamin C and xylazine at 30 min | 24.8 ±2.46 | 35.3±6.57 | 42.34 |
| Group F | | | |
| Vitamin C and xylazine at 60 min | 8. 5±1.25 | 29.6±1.03 | 248.24 |

SD standard deviation
MST mean sleeping time
NSO no sleep observed

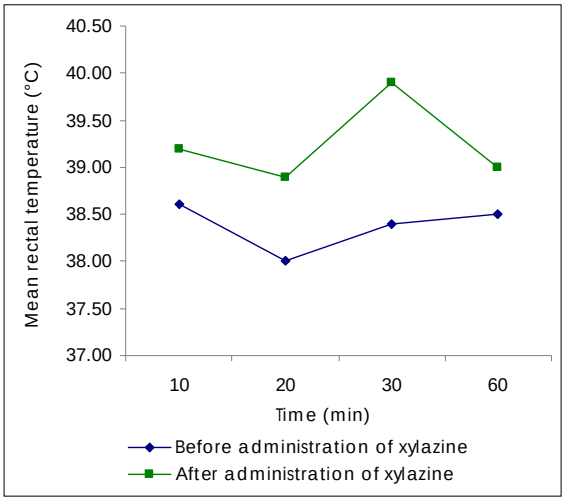


Figure 1
Mean rectal temperature changes before and after xylazine treatment in rabbits

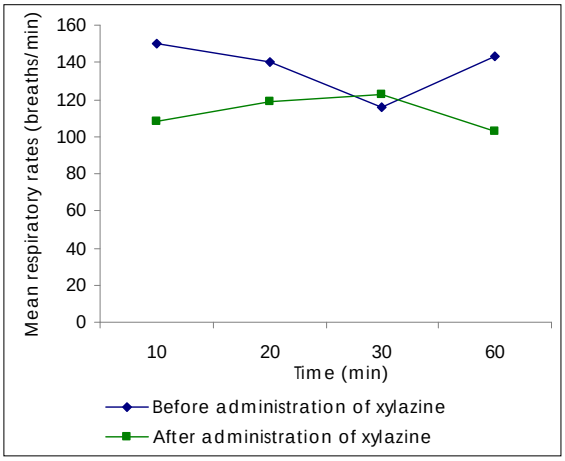


Figure 2
Mean respiratory rate changes before and after xylazine treatment in rabbits

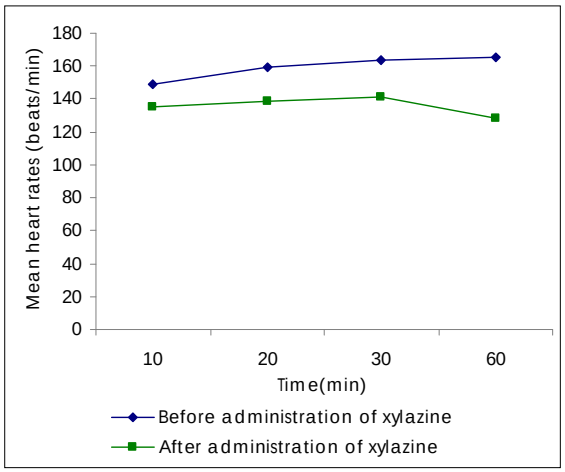


Figure 3
Mean heart rate changes before and after xylazine treatment in rabbits

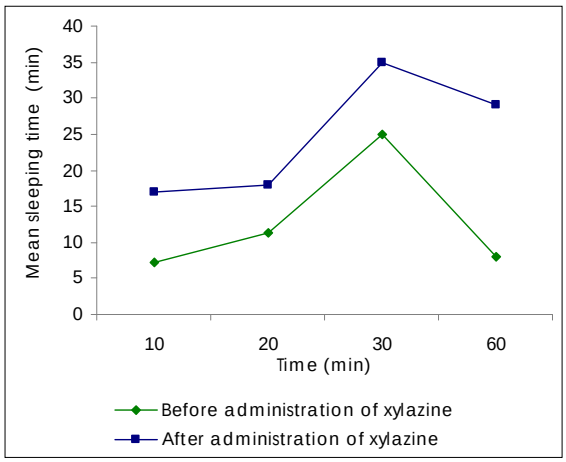


Figure 4
Mean sleeping time changes before and after xylazine treatment in rabbits

## Discussion

The results of this study suggest that vitamin C pre-medication prior to xylazine anaesthesia affected the onset and duration of xylazine anaesthesia in rabbits. There was a slight increase in temperature when xylazine was used alone. This confirms earlier reports observed in some animals such as cattle and sheep (23). In contrast, Kerr *et al.* (13) reported hypothermia in horses when xylazine was administered. The administration of vitamin C alone increased the temperature to higher levels than when xylazine was used alone. This may be as a result of the modulating influence of vitamin C which Ezenwanne and Anuka (6) reported may lead to behavioural changes and Hasar and Najafpour (11) and Sjostrand (20) opined may cause sleep disturbances, headaches and intestinal upsets, all of which may culminate in a rise in temperature as observed in cats (11). These effects may be a result of the membrane stabilising effect of vitamin C (22), thus preventing further movement of xylazine in and out of the brain.

The respiratory rate increased when xylazine was used alone. The use of vitamin C alone prior to xylazine administration resulted in a greater depression in respiratory rate. It is possible that vitamin C-induced central nervous

system depressant activity may be responsible for the decrease in respiratory rate.

The heart rate decreased significantly after the administration of xylazine alone. However, vitamin C, in combination with xylazine, provided a lesser drop in heart rate than xylazine alone. When the interval between vitamin C and xylazine administration was increased, the sleeping time also increased. This may be so because of the principal potentiating effects of xylazine which has been shown to develop between 10-15 min after intramuscular administration (10).

## Conclusions

This study showed that vitamin C alters the clinical parameters as well as prolonged the sleeping duration of rabbits anaesthetised with xylazine. Vitamin C prolonged the duration of anaesthesia which is particularly advantageous against anaesthetic toxicity, especially if higher doses of xylazine are used for prolonged surgical exploration. From these results, it is therefore recommended that vitamin C pre-medication at 120 mg/kg *per os* should be administered 60 min prior to xylazine anaesthesia in rabbits. However, the dose response with different doses of vitamin C pre-medication on xylazine anaesthesia will require further investigations.

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## Conflict of interest

The authors have no conflict of interest to disclose.

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