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RESEARCH ARTICLE

Effects of Short-Acting Anaesthetics on Haemodynamic Function as Determined by Doppler Ultrasonography in Rabbits

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Summary

This study was carried out to determine the effects of short-acting anaesthetics on haemodynamic function determined by Doppler ultrasonography. Prior to anaesthesia, Doppler parameters [peak systolic blood flow velocity (psBFV), end-diastolic blood flow velocity (edBFV), minimum diastolic blood flow velocity (mdBFV) and resistive index (RI)] were obtained from the right common carotid artery (CCA), abdominal aorta (AA) and right kidney in New Zealand rabbits (n=24). Animals were then divided into 3 groups to be anaesthetized with propofol (Group P), 2.5% thiopental sodium (Group T) and xylazine/ketamine HCl (Group XK). During anaesthesia the same Doppler measurements were made. Compared with baseline values, psBFV obtained from CCA increased in Group P (P<0.02) and insignificantly changed in Groups T and XK. psBFV measured from AA in Groups T and XK decreased as compared with baseline values. In the right kidney, psBFV in Group P and edBFV in Group T increased, whereas RI value in Groups T and XK decreased. In conclusion, comparing baseline values, propofol anaesthesia did not significantly alter RI measured from CCA and kidney and blood flow velocities measured from AA. The data suggest that propofol anaesthesia in rabbits results in minimal changes in Doppler parameters.

Keywords: *Short-acting anaesthetic, Haemodynamic function, Doppler ultrasonography, Rabbit*

Kısa-Etkili Anesteziklerin Tavşanlarda Hemodinamik Fonksiyonlara Etkilerinin Doppler Ultrasonografi ile Değerlendirilmesi

Özet

Bu çalışma, kısa etkili anesteziklerin Doppler ultrasonografi ile saptanan hemodinamik fonksiyonlara etkilerini belirlemek amacıyla yürütüldü. Anestezi öncesi Yeni Zelanda tavşanlarının (n=24) sağ arteria carotis communis (CCA), aorta abdominalis (AA) ve sağ böbreğinden Doppler parametreleri [en yüksek sistolik kan akım hızı (psBFV), diyastol sonu kan akım hızı (edBFV), en düşük diyastolik kan akım hızı (mdBFV) ve rezistif indeks (RI)] elde edildi. Hayvanlar propofol (Grup P), %2.5 tiyopental sodyum (Grup T) ve ksilazin/ketamin HCl (Grup XK) ile anestezi altına alınarak, üç grup oluşturuldu. Anestezi sırasında aynı ölçümler tekrarlandı. Bazal değerler ile karşılaştırıldığında CCA'dan elde edilen psBFV Grup P'de yükseldi ve Grup T ve XK'daki değişim önemsizdi. Grup T ve XK'da AA'dan ölçülen psBFV bazal değerler ile kıyaslandığında azaldı. Sağ böbrekte, Grup P'nin psBFV ve Grup T'nin edBFV değerleri artarken, Grup T ve XK'da RI değeri azaldı. Sonuç olarak, propofol anestezisi bazal parametreler ile karşılaştırıldığında, CCA'deki RI, intrarenal RI ve AA'daki kan akımı hızlarını önemli ölçüde değiştirmediği tespit edildi. Veriler propofol anestezisinin tavşanlarda Doppler parametrelerini minimal düzeyde etkilediğini göstermektedir.

Anahtar sözcükler: *Kısa-etkili anestezik, Hemodinamik fonksiyon, Doppler ultrasonografi, Tavşan*

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INTRODUCTION

Vascular Doppler ultrasonography (DU) is commonly employed in small animal medicine [1,2]. Doppler ultrasonography provides real-time anatomic and dynamic vascular flow data, including peripheral vascular resistance [3], which makes identifying pathological changes possible [4,5]. Vascular impedance data cannot be obtained from absolute velocity. Vascular resistance indices, such as resistive index (RI) and pulsative index (PI), have therefore been developed to enable the evaluation and comparison of Doppler wave forms [3]. Blood flow is determined without sedation in human medicine. However, prior to imagining, sedation may be required for restraining in veterinary medicine because poor patient cooperation, high respiratory and heart rates and voluntary movement could interfere with the outcome, especially in cases that require detailed investigations such as abdominal vascular ultrasonography [2,6].

Propofol is a unique non-barbiturate, non-steroid, short -acting general intravenous anaesthetic agent used to provide sedation and to induce and maintain anaesthesia [7]. It is highly lipophilic and quickly metabolized, largely to inactive glucuronide conjugates. Thiopental was introduced into veterinary practice in 1937. Over the next 70 years it has become the most widely used induction agent, particularly for cats and dogs [8]. Alike propofol, thiopental is associated with a rapid smooth induction and a rapid recovery [9]. Equivalent cardiovascular and respiratory effects are produced by both anaesthetic agents [8]. The combination of ketamine - xylazine has also been used for several species for many years and is still popular for laboratory animal medicine [10]. It is associated with a rapid onset, good or excellent sedation over 1-2 h, excellent analgesia lasting 15 to 30 min and a smooth recovery [11].

Anaesthetic agents may also alter systemic and renal haemodynamics through affecting vascular resistance [12,13] in DU imaging. A combination of atropine, diazepam, acepromazine, and ketamine was shown to reduce the renal RI in healthy dogs [12]. In cats, sedation with a combination of atropine, acepromazine, and ketamine did not alter the renal RI [14]. However, anaesthesia with isofluorane increased both the renal RI and PI in cats [15]. In the literature, there are no sufficient data from comparison of short-acting anaesthetic agents' effects on DU parameters.

This study was conducted to evaluate the effects of propofol, thiopental sodium and xylazine/ketamine anaesthesia, frequently used in veterinary medicine and experimental research, on blood flow velocities and resistive index values obtained from the common carotid artery (CCA), abdominal aorta (AA) and kidney using duplex DU in rabbits.

MATERIAL and METHODS

Animals, Management, and Experimental Groups

Twenty-four male New Zealand White rabbits were used. Mean body weight was 3.8±0.59 kg (mean ± SD) and age ranged between 14 and 27 weeks with a mean of 19±6 weeks. Animals were housed in individual cages on dust-free wood shavings in a room with temperature of 21-24°C and a 12:12 h light-dark cycle. The rabbits were fed a commercial pelleted diet and given water *ad libitum*.

All animals were adapted to housing conditions for at least 7 days. After obtaining baseline Doppler parameters, rabbits were randomly divided into one of 3 groups: Group P (propofol, Pofol®, Sandoz, Turkey), Group T (thiopental sodium, Pentothal®, Abbott, Italy), and Group XK (xylazine/ketamine HCl, Rompun®, Bayer, Germany; Ketasol®, Interhas, Turkey). The experimental protocol was approved after Institutional Ethics Committee, and animals were handled in compliance with the Principles of Laboratory Animal Care.

Anaesthesia and Ultrasonography Protocols

Heart and respiratory rates were monitored before and during the anaesthesia provided by each agent. A venous catheter was inserted in the lateral ear vein. Animals in Groups P, T, and XK were anaesthetized with propofol (8 mg/kg ± 0.76, IV), 2.5% thiopental sodium (20 mg/kg ± 1.24, IV), and xylazine/ketamine HCl (4 mg/kg plus 10 mg/kg, IV), respectively. The dosages were determined to achieve a stable and light level of anaesthesia, characterized by good muscle relaxation and hypnosis with stable baseline vascular variables. Anaesthesia lasted for 15 min.

Before assigning rabbits into experimental groups, the ventral part of the cervical region and the right and ventral abdominal regions were shaved. After gel application, DU measurements of the right CCA were made in dorsal recumbence, whereas DU measurements of the AA and right kidney were made in lateral recumbence. Measurements made from CCA were peak-systolic blood flow velocity (psBFV), end-diastolic blood flow velocity (edBFV) and RI values; those made from AA were minimum-diastolic blood flow velocity (mdBFV), psBFV, edBFV; and those made from the arcuate artery and/or interlobar artery of the kidney were psBFV, edBFV and intrarenal RI value. The RI value was calculated using the equation: [(psBFV-edBFV)/psBFV]. The same DU measurements were obtained from the same locations while rabbits were under anaesthesia.

A linear probe with a multifrequency feature (7.5-10 MHz) was used in DU (Esaote AU5, Genoa, Italy). High pulse repetition frequency was used during examination of the AA and right CCA. The probe was positioned over the ventral cervical region in order to measure the vascular

parameters of the right CCA. Doppler spectra were obtained on the transversal and longitudinal planes for right CCA. At examination of the AA, the probe was placed over the abdominal region at the caudal end of the thorax, where the pulsating aorta can be seen longitudinally just ventral to the vertebrae, to the left of and parallel to the adjacent caudal vena cava, touching each other. The probe position was adjusted until distinct parallel vessel walls of the AA and right CCA were visible. These vascular structures were then evaluated with colour DU. Gain and flow sensitivity measurement adjustments were maintained at the optimal level so as to fill the vessel lumen during systole but not to produce artifact in neighbouring tissues. The Doppler sample volume was placed centrally within the vessel and the sample volume cursor was adjusted to align with the vessel walls and blood flow. An angle between 45° and 60° was consistently achieved between the vessel and ultrasound beam. Once the sample volume had been correctly positioned, Doppler examinations were carried out in duplex Doppler mode. Recorded velocity spectra were assessed for quality by clarity of the visual and audible signals and then stored to measure psBFV, mdBFV and edBFV. The RI for CCA was derived from psBFV and edBFV.

For renal DU, kidney morphology was evaluated at the dorsal and sagittal planes during ultrasonography. The wall filter and sample volume were maintained at a minimum level during renal DU. Measurements were taken at a 60° angle. Colour DU was used to visualize the intrarenal vasculature (arcuatae and interlobular arteries). Thus, spectral samples were obtained from one of these arteries using duplex Doppler. Doppler flow spectra recorded for analysis consisted of at least three consecutive similar-patterned wave forms. The mean intrarenal RI in the right kidney was determined by averaging nine Doppler waveforms from the interlobar or arcuate arteries at three separate locations (three waveforms at cranial, middle, and caudal poles) as previously described.

Statistical Analysis

Velocities, RI values, heart and respiratory rates differences between groups were analyzed using one-way ANOVA (SAS 1999, *User's Guide* Statistics, Version 8, Statistical Analysis System, SAS Inst. Inc., Cary, USA). Data were presented as means with pooled SEM. Differences were considered significant at P<0.05.

RESULTS

Heart and Respiratory Rates

All anaesthetic agents produced a rapid and smooth induction of anaesthesia in all animals. These agents provided adequate hypnosis and relaxation for DU measurements. Heart and respiratory rates are presented in *Table 1*. Heart rate decreased in the Groups T and X/K (P<0.02) but did not change in the Group P when compared with baseline values. Respiratory rate decreased during anaesthesia regardless of the agents (P<0.001).

Flow Patterns

Continuous forward flow was observed in the AA. Colour stemming from blood flow uniformly filled the lumen. Dark colour was observed inside the lumen at diastole, and during systole this turned into a bright colour indicating high velocity. Despite flow being constant in the right CCA, the colour in the lumen centre was lighter and brighter compared to that in the periphery close to the vascular wall. At sagittal imaging in the right kidney, good quality colouring was observed in the renal cortex and interlobar vascular structures. Following the administration of anaesthetics, no noteworthy change in color DU of the AA and right CCA and kidney was observed.

The AA had a plug flow velocity profile and its waveform was a high-resistance flow pattern. It had a sharp systolic peak with a large and clear spectral window. The velocity distribution was narrow. The systolic peak was followed by a retrograde flow wave, followed by a forward flow wave (three-phasic flow wave) *(Fig 1)*. In the right CCA the systolic peaks were sharp without a clear spectral window. There were notable double systolic peaks. The systolic peak was not followed by retrograde wave (intermediate resistance flow pattern). In mid-diastole a forward flow wave with a higher velocity was visible. Then, flow velocity declined again *(Fig 2)*. In intrarenal vessels, spectral wave forms had a blunted parabolic flow velocity profile. This continuous forward flow was a low resistance flow pattern. The systolic peaks were broader with a very small, closed spectral window. In conscious rabbits, there were early systolic peaks *(Fig 3A)*, which continued in Group P but discontinued in Groups T and XK. End-diastolic blood flow velocity was clearly increased in Group T *(Fig 3B)*.

Table 1. *Effect of different anaesthetics on heart and respiratory rates*
Tablo 1. *Farklı anesteziklerin kalp ve solumun oranlarına etkisi*

Variable	Groups				SEM	P<
	B	P	T	XK		
Heart rate	234[a]	247[a]	163[b]	179[b]	3.0	0.02
Respiratory rate	72[a]	42[b]	37[b]	38[b]	1.2	0.001

B = Baseline value; P = Propofol; T = Thiopental sodium; XK = Xylazine HCl plus Ketamine HCl Different superscripts within the same row significantly differ (P<0.05)

Blood Flow Velocities and Resistive Index Values

Compared to baseline, psBFV in the right CCA increased in Group P (P<0.02), whereas remained unchanged in Groups T and XK *(Table 2)*. The RI value obtained from CCA however did not change in Group P, but decreased in Group T and XK (P<0.0001). The edBFV value obtained from CCA was not affected by the anaesthetic agents. The psBFV obtained from AA decreased in Groups T and XK (P<0.0001), whereas that in Group P did not change in comparison with baseline *(Table 2)*. mdBFV and edBFV values measured from AA were comparable within the groups, but both did not change comparing with baseline. The psBFV value from the RK in Groups T and XK did not

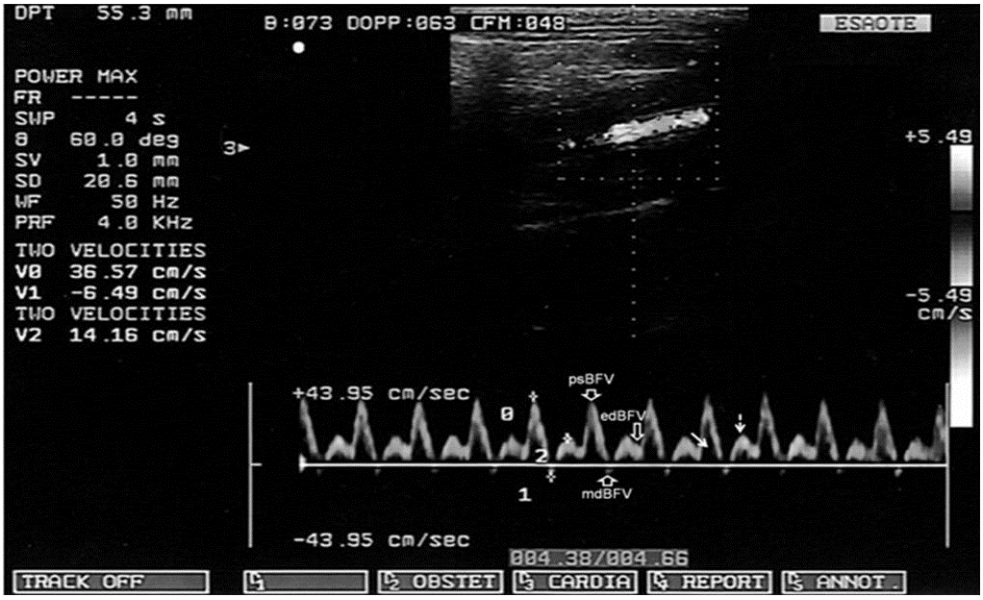

Fig 1. Duplex Doppler ultrasound image of the abdominal aorta, peak systolic blood flow velocity (psBFV), minimum diastolic blood flow velocity (mdBFV) and end-diastolic blood flow velocity (edBFV) in longitudinal plane. Plug flow velocity profile the systolic peaks (empty arrow) are sharp, and white arrow represent a clear spectral window. The systolic peak is followed by a retrograde flow wave (mdBFV) in early diastole with a high-resistance flow pattern and the retrograde flow wave is followed by a forward flow wave (white broken arrow)

Şekil 1. Aorta abdominalisin duplex Doppler ultrasonografik görüntüsü, en yüksek sistolik kan akım hızı (psBFV), en düşük diyastolik kan akım hızı (mdBFV) ve diyastol sonu kan akım hızı (edBFV). Plug akım hız grafiğinde sistolik tepe (boş ok) keskin ve beyaz ok açık spektral pencereyi göstermektedir. Sistolik tepeyi yüksek dirençli akım örneğinde görülen erken diyastoldeki ters akım dalgası (mdBFV) takip etmekte ve ters akım dalgasını, ileri akım dalgası (beyaz kesik ok) izlemektedir

Table 2. *Effect of different anaesthetics on the Doppler parameters of the right common carotid artery (CCA), abdominal aorta (AA) and right kidney (RK)*

Tablo 2. *Farklı anesteziklerin sağ ateria carotis communis (CCA), aorta abdominalis (AA) ve sağ böbreğin (RK) Doppler parametrelerine etkisi*

Variables [2]		Groups[1]				SEM	P<
		B	P	T	XK		
CCA	psBFV	31.6^{b}	42.3^{a}	26.4^{b}	30.0^{b}	3.1	0.02
	edBFV	10.4	12.0	12.7	12.7	1.0	0.29
	RI	0.67^{a}	0.71^{a}	0.54^{b}	0.58^{b}	0.02	0.0001
AA	psBFV	49.3^{a}	51.7^{a}	27.4^{b}	32.4^{b}	3.6	0.0001
	mdBFV	7.4^{ab}	10.6^{a}	5.8^{b}	6.9^{ab}	1.2	0.07
	edBFV	12.5^{ab}	15.6^{a}	9.5^{b}	9.4^{b}	1.6	0.04
RK	psBFV	30.1^{b}	41.8^{a}	31.6^{b}	30.0^{b}	3.3	0.002
	edBFV	13.8^{b}	17.5^{ab}	20.9^{a}	16.3^{ab}	2.0	0.02
	RI	0.54^{a}	0.58^{a}	0.34^{c}	0.45^{b}	0.03	0.0001

[1] *B = Baseline values; P = Propofol; T = Thiopental sodium; XK = Xylazine HCl plus Ketamine HCl Different superscripts within the same row significantly differ (P<0.05)*

[2] *psBFV = peak-systolic blood flow velocity (cm/s); edBFV = end-diastolic blood flow velocity (cm/s); RI = resistive index; mdBFV = minimum-diastolic blood flow velocity (cm/s)*

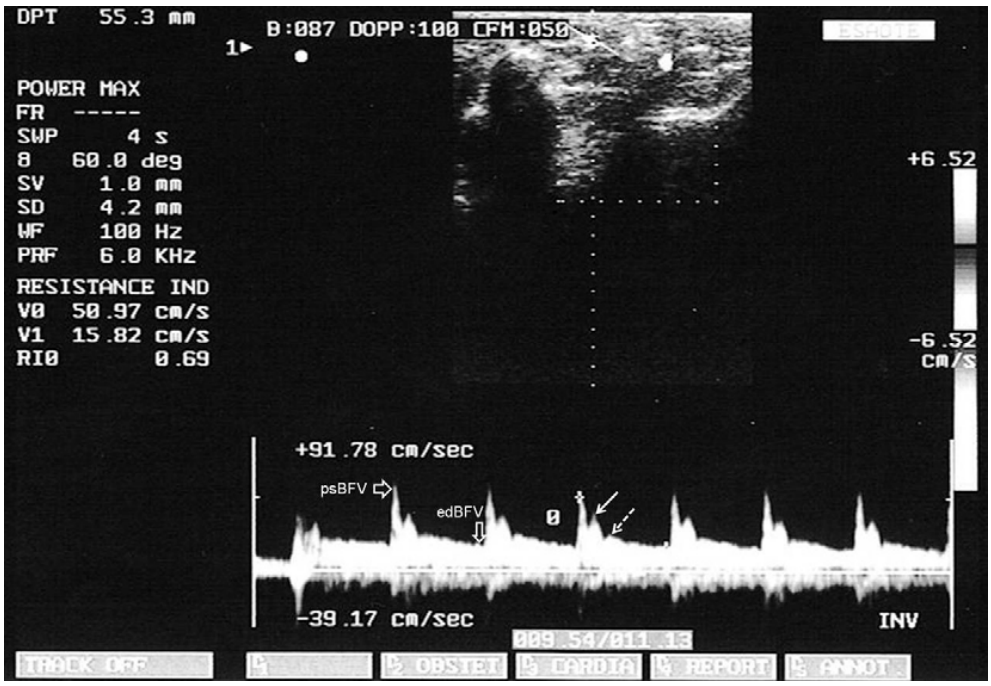

Fig 2. Duplex Doppler ultrasound image of the right common carotid artery, exhibiting peak systolic blood flow velocity (psBFV) and end-diastolic blood flow velocity (edBFV) in transversal plane. The systolic peaks are sharp. White arrow indicates second systolic peaks. The spectral window is closed. In mid-diastole a forward flow wave with a higher velocity (broken white arrow) is visible. The flow velocity then falls again

Şekil 2. Transversal planda sağ arteria carotis communisin duplex Doppler ultrasonografik görüntüsü, en yüksek sistolik kan akım hızı (psBFV), en düşük diyastolik kan akım hızı (mdBFV). Sistolik tepe keskin bir şekilde sonlanmaktadır. Beyaz ok, ikinci sistolik tepeyi işaret etmektedir. Spektral pencere kapalıdır. Diyastolde hızın daha da yükselmesine bağlı olarak ileri akım dalgası (kesik beyaz ok) görülmektedir. Sonra akım hızının tekrar düştüğü izlenilmektedir

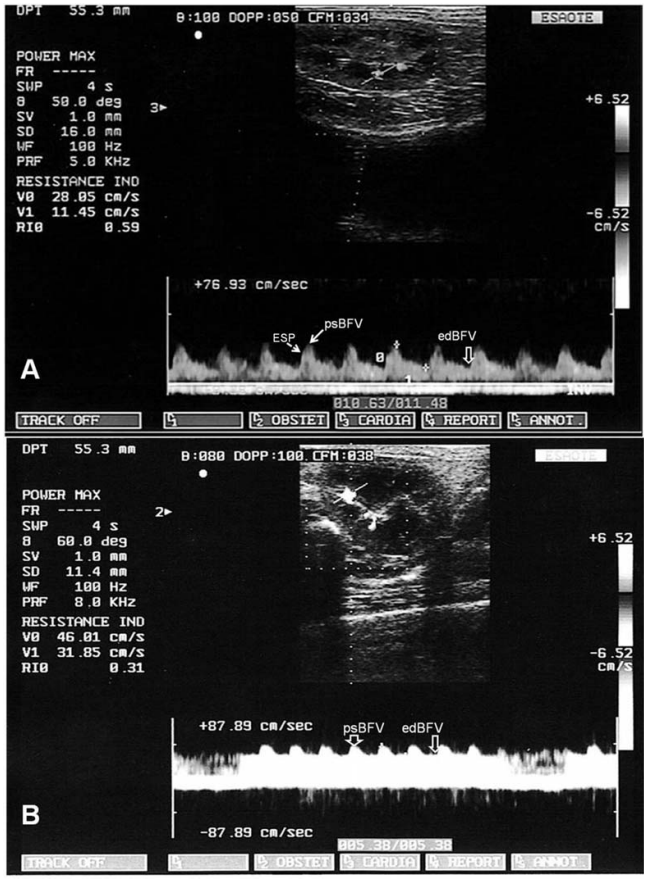

Fig 3. Duplex Doppler ultrasound images of the right kidney, exhibiting peak systolic blood flow velocity (psBFV) and end-diastolic blood flow velocity (edBFV) in sagittal plane. **A.** In a consciouss rabbit low resistance flow pattern is indicated by continuous, broad systolic peaks and diastole with gradually decreasing edBFV. Early systolic peaks (ESPs, broken arrow) observed immediately before the systolic peak. **B.** Broading in psBFV and increased edBFV in a rabbit from Group T. Early systolic peaks are not apparent

Şekil 3. Sagittal planda sağ böbreğin duplex Doppler ultrasonografik görüntüsü, en yüksek sistolik kan akım hızı (psBFV), diyastol sonu kan akım hızı (edBFV). **A.** Bilinçli bir tavşanda düşük dirençli akım örneği, devamlı, köşeli sistolik tepe ve dereceli olarak azalan edBFV diyastol ile görülmektedir. Sistolik tepeden hemen önce görülen erken sistolik tepeler (ESPs, kesik ok) izlenmektedir. **B.** Grup T'deki bir tavşanda psBFV'de kütleşme ve artmış edBFV görülmektedir. Erken sistolik tepeler izlenilmemektedir

change, whereas increased in Group P compared to baseline *(Table 2)*. Only in Group T, edBFV value for kidney increased compared to baseline (P<0.02). The intrarenal RI value in Group P remained unchanged and decreased in Groups T and XK, at a greater extent in Group T than Group XK *(Table 2)*.

DISCUSSION

Doppler Ultrasonography is a non-invasive and accurate method for evaluating blood flow invariety of vessels [16]. Percutaneous ultrasonographic examination allows immediate visualization of vascular effects in awake and anesthetized animals. It is also comparable with perivascular ultrasonographic flow probes as a non-invasive method [17]. Anaesthetic agents may change systemic and renal haemodynamics and subsequently affect vascular resistance. Doppler flow technology using high-resolution vessel images combined with haemodynamic monitoring can provide extensive data on cardiovascular effects of drugs [18]. Administration of propofol has limited effects on heart contractility [19], although it induces arterial hypotension primarily by reducing vascular tone and venous return [20] as well as producing peripheral vasodilatation [17]. However, Nakamura et al.[21] demonstrated that clinically relevant concentrations of propofol did not have direct vasodilator effects. On the other hand, Baumgartner et al.[22] indicated that injections of propofol (8 mg/kg^{-1}) to rabbits produced an immediate, transient vasodilatation, a significant decrease in ventricular performance and an increase in peripheral vascular resistance. These were accompanied by increases in psBFV and RI obtained from CCA and AA and by decrease in edBFV obtained from AA. Similarly, psBFV obtained from CCA in Group P increased as compared to both baseline and Groups T and XK (*Table 2*; P<0.02). A correlation can be established between propofol increasing blood flow and peripheral vascular resistance in the CCA [17,22]. In the present study, psBFV, edBFV and RI obtained from CCA increased during injection of propofol *(Table 2)*. Calculation of the RI and PI permits indirect determination of changes in systemic vascular resistance within the distribution area of measured vessels [18]. Increased arterial pressure not only increases the force that pushes blood through the vessels but simultaneously distends the vessels, resulting in lowered vascular resistance. This may also account for a slight elevation in RI obtained from CCA. However, increases in psBFV, mdBFV and edBFV obtained from AA in Group P were insignificant *(Table 2)*. Despite the hypotensive and vasodilatory effects of propofol, which cause a decrease in velocity values, blood flow indices obtained from AA in Group P was minimally affected.

It is known that the rapid intravenous injection of thiopental causes a decrease in blood pressure even in normovolemic animals. After an initial fall, the blood pressure returns to approximately the preanaesthetic level, albeit often with a persistent tachycardia. Thiopental seems to have a direct depressant effect on the myocardium and under certain circumstances may produce cardiac arrhythmias. Thiopental modifies the vasomotor response to increases in intrathoracic pressure (Valsalva manoeuvre). In the presence of thiopental, excessively vigorous controlled respiration will produce a fall in arterial pressure by increasing mean intrathoracic pressure. Induction doses of thiobarbiturates produce a central and peripheral cardiovascular depression, and consequently lower blood pressure [12]. Administration of xylazine - ketamine results in large variations in cardiac function [23,24] and contractility [25]. Combination of xylazine-ketamine (5 mg/kg - 35 mg/kg) also induces a drop of blood pressure, heart rate, and respiratory rate in rabbits [26]. In a study involving rabbits the intravenous injection of xylazine-ketamine (0.4 mg/kg - 4 mg/kg, IV) increased vessel diameter of CCA and AA and decreased mean volumetric flow in CCA [27]. Although there was a change in RI value in CCA, psBFV obtained from AA decreased. In our study, psBFV obtained from right CCA in both Group T and Group XK did not change, but psBF obtained from AA decreased (P<0.001; Group T: 49%, Group XK: 34% *(Table 2)*. This could be related to the negative effect of thiopental and ketamine-xylazine on volumetric blood flow and blood pressure. In comparison with baseline values, RI obtained from CCA exhibited a significant decrease in Groups T and XK (P<0.0001). However, the changes in psBFV and edBFV obtained from CCA in Groups T and XK were insignificant *(Table 2)*.

Intrarenal RI is calculated from blood flow velocities in vessels during the cardiac cycle and reflects renal haemodynamics [28]. This index is particularly useful in diagnosing and evaluating obstructive uropathy [29-31] and is correlated with renal disease severity [28]. Intrarenal RI and heart rate are negatively correlated in humans [32]. However, Novellas et al.[3] emphasized that some animals had a decreased heart rate during sedation, but relationship between renal RI and heart rate was not determined before or during sedation. No relationship was detected in our study.

Although propofol has been reported to affect haemodynamics by negative inotropy and vasodilation, its effect on renal blood flow in healthy subjects is minimal [33,34]. While propofol may reduce renal function, similar to other anaesthetic compounds, in healthy animals, the depression in renal blood flow appears to be less than that during anaesthesia induction using volatile anaesthetics [35]. Although there was an increase in both psBFV (145%) and edBFV (127%) in Group P, intrarenal RI did not change in comparison with baseline values, suggesting that the haemodynamic effects of propofol anaesthesia in rabbits is not detrimental enough to alter intrarenal RI. Changes in psBFV were insignificant in

Groups T and XK, while the rise in edBFV in both groups was remarkable (Group T: 151%, Group XK: 121%). In Group T in particular, the significant increase in edBFV and broading in systolic peaks shows that thiopental may cause a less resistant flow in rabbits *(Fig 3)*.

In conclusion, while propofol induces no significant change in RI obtained from CCA and blood flow velocities obtained from AA and intrarenal RI, thiopental and xylazine/ketamine anaesthesia significantly reduced RI value obtained from CCA as well as intrarenal RI and psBFV and edBFV obtained from AA. The results of this study indicate that propofol has a minimal effect on Doppler parameters in anaesthesia/Doppler procedures in rabbits.

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