

## Evaluation of the Effect of Ampicillin on the Haematological Parameters of Rabbits (*Oryctolagus cuniculi*) Following Thiopentone Sodium-induced Anaesthesia

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**Abstract:** The effect of ampicillin on haematological parameters of rabbits following thiopentone sodium anaesthesia was investigated. Six New Zealand white rabbits weighing 3-5kg, aged 6-7 months were administered ampicillin (10 mg/kg) *via* the left jugular vein and blood samples were collected *via* the contralateral vein at 0, 1, 3, 6, 9, 12 and 24 hours postdrug administration. Four weeks later, the rabbits were administered same dose of ampicillin immediately following recovery from thiopentone sodium (20 mg/kg) anaesthesia. Blood samples were collected as above and packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC), haemoglobin (Hb), mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined. The results showed significant decrease ( $P < 0.05$ ) in values of PCV, Hb, WBC and RBC and increase in MCV. However, PCV, Hb, WBC, RBC, MCV and MCH were decreased when ampicillin was administered after recovery from thiopentone anaesthesia. While, most of the haematological changes peaked at the third hour post ampicillin administration, the alterations were mostly severe at the sixth hour when both drugs were administered accordingly. It can be concluded that administration of thiopentone sodium increase the effect of ampicillin on haematological parameters of rabbits.

**Key words:** Ampicillin • Anaesthesia • Haematological parameters • Rabbits • Thiopentone sodium

### INTRODUCTION

The use of antibiotics in both pet and food-producing animals has led to improved health status, enhanced animal productivity, declined disease incidence and reduced morbidity and mortality in humans and animals. This has resulted in the production of adequate quantities of nutritious, high-quality and affordable food for human consumption [1]. Antibiotics are particularly vital in mitigating infections prior to development of specific immunity, especially in debilitated, stressed animals or where the infection is severe [2]. The prophylactic use of antibiotics is recommended when surgical operation are performed in contaminated areas or infection is present in the patient undergoing surgical operation or in a patient who had undergone surgery where chances of complications of peritonitis threatening the life of the patients could not be completely avoided

[3-5]. One of the most common reasons that necessitates antimicrobial administration is for perioperative prophylaxis. The goal of prophylactic antimicrobial administration is to reduce the chances of postoperative infection especially at the surgical site, thereby lowering the rate of mortality and morbidity while avoiding the cost of full antibiotic regime [6, 7]. However, due to the unavoidable inadequate hygienic operating conditions often encountered during emergency ambulatory surgeries, several veterinary surgeons choose to administer perioperative antimicrobials [8, 9]. The major risk factors that have been shown to be associated with surgical wound infections includes operative factors such preoperative preparation of the surgical site, operation technique, surgeon's skills, type and duration of surgery and host associated factors such as advance in age, immune status of the host, obesity, cancer, malnutrition and diabetes [10].

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Ampicillin, semi-synthetically derived from penicillin, is an active broad spectrum antibiotic. It belongs to the  $\beta$ -lactam group penicillin antibiotics. Its only difference from penicillin is the existence of an amino group which enhance penetration via the outer membrane of gram-positive and some gram-negative bacteria. Its effect is either bactericidal or bacteriolytic and its actions are most marked during the active growth phase [11]. Ampicillin is often used to prevent and treat infections found in cuts and wounds, the mouth, the upper respiratory system and the bladder. It has comparatively few side effect, permitting it to be used preferentially over other agents such as chloramphenicol or tetracycline when a broad spectrum antibiotics is desired [12].

Thiopentone sodium is a general anaesthetic agent and it is commercially available in sealed ampoules as a powder buffered with sodium carbonate [13]. Thiopental is an ultra-short-acting barbiturate which remains the standard against which other anaesthetic agents are compared [14]. Anaesthesia is a necessary part of veterinary medicine for restraint, elimination of pain sensation during surgical and medical procedures and for appropriate stabilization of the critically ill animal before surgery [15]. Rabbits have gain general acceptance as laboratory animals in experimental surgeries and increasingly as pets. Thiopentone sodium injection for the induction of anaesthesia appears to be the most common method employed in rabbits [16].

Haematological parameters on the other, hand play important roles in clinical diagnosis of both infectious and non-infectious diseases. They are crucial in assessing responses following treatment and in prevention of diseases. Alterations in haematological parameters are important in evaluating the responses of animals exposed to various physiologic conditions [17].

Even though an increasing volume of data is continuously generated from numerous studies investigating the use of prophylactic antibiotics in surgery [18] and much data is available in human medicine, objective scientific studies which evaluate the use of perioperative prophylaxis in veterinary medicine are still limited and more studies are needed [7]. Furthermore, there is paucity of information on the prophylactic use of antibiotics in surgery or established guidelines on indications for surgical antibiotic prophylaxis and its benefits in Nigerian Veterinary practice [18]. Studies of this nature could provide a valuable information for both clinicians and researchers. The effect of ampicillin and the consequent effect of the administration of both agents on the haematological parameters of rabbits is unknown. We

hypothesized that the administration of ampicillin following thiopentone sodium anaesthesia in rabbits could further exacerbate the haematological alterations associated with ampicillin. Therefore, the aim of this study was to investigate the effect of ampicillin following recovery from thiopentone sodium anaesthesia on haematological parameters of rabbits.

## MATERIALS AND METHODS

The 6 clinically healthy male New Zealand white male rabbits of 6 months old, weighing  $1.5 \pm 0.2$  kg used in this experiment were procured from a commercial farmer. The rabbits were housed in well-spaced wire gauze cages which were bedded with saw dust for 3 weeks to acclimatize to handling and changes in housing conditions. Commercial pellets and water were provided *ad libitum*. Fresh vegetables and hay were also supplemented.

**Drugs:** Ampicillin sodium B.P equivalent to 500 mg anhydrous ampicillin (Cayman chemical, USA) was acquired from Biocom Nigeria. Thiopentone sodium (Thiopentone®, Rotex medica, Germany) was acquired from Biocom Nigeria. They were reconstituted with sterile distilled water to the required concentrations as recommended by the manufacturer. Both drugs were used immediately after reconstitution according the manufacturer's instruction.

**Ethical Statement:** The experiment was performed in strict compliance with the guidelines provided by the Animal ethical committee, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. All animals were carefully and humanely handled to minimize avoidable stress throughout the study. Samples were aseptically and humanely collected throughout the study.

**Drug Administration and Sampling:** The 6 rabbits were shaved at the neck region to expose the external jugular area. Ampicillin sodium was then administered at the dose of 10 mg/kg body weight intravenously *via* the left jugular vein. Blood samples were collected *via* the contralateral vein at time 0 (immediately prior to drug administration) and at 1, 3, 6, 9, 12 and 24 h post drug administration into bottles with Ethylene diamine tetra acetic acid (EDTA) for the determination of Packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC) and red blood cell (RBC) counts. The PCV was determined by the microhaematocrit method. Haemoglobin (Hb)

concentration was determined by the cyanmethaemoglobin method. White blood cell and red blood cells (RBC) were counted using a haemocytometer [19]. Mean cell volume (MCV), Mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were determined from RBC, PCV and Hb.

After a period of 4 weeks, the 6 rabbits were administered freshly prepared thiopentone sodium (Thiopentone®, Rotex medica, Germany) at 20 mg/kg body weight intravenously *via* the left external jugular vein using 23G needle. Immediately following recovery from the anaesthesia, the rabbit were administered ampicillin at 10mg/kg body weight via the left external jugular vein. Blood samples were similarly collected in the same manner as described above. Again the PCV, Hb, WBC, RBC, MCV, MCH and MCHC were determined using the same techniques as above.

**Data Analysis:** All data are presented as mean  $\pm$  standard deviation. Changes in packed cell volume (PCV), Hb, WBC and RBC over time were analysed by comparing the means obtained at the different time points after anaesthesia with their respective baseline mean values using one-way analysis of variance (ANOVA) in SPSS version 20. Also the mean values of PCV, Hb, WBC and RBC obtained from the first experiment were compared with the mean values obtained in the second experiment using ANOVA. Statistical significance were determined at  $P < 0.05$ . All figures are drawn using GraphPad Prism 6 software.

## RESULTS

**Mean Packed Cell Volume (PCV):** The mean PCV values from the rabbits for both the first and second experiment is presented in Fig. 1. Administration of ampicillin alone at the dose of 10 mg/kg body weight as performed in the first experiment was observed to induce a considerable decrease in PCV from the baseline value of 30% to 27.2% at the first hour post ampicillin administration. The value further declined to 25.2% at the 3<sup>rd</sup> h post drug administration (PAA). However, the PCV value was increased at the 6<sup>th</sup> h PAA to 29.6% and then steadily decreased significantly ( $p < 0.05$ ) to 25% at the 24<sup>th</sup> h PAA. In the second experiment, the combined administration of thiopentone (at 20 mg/kg body weight) prior to ampicillin was observed to induce a steady decrease through the 6<sup>th</sup> h, where the PCV value was 25.2%. The PCV value continued declining towards the 24<sup>th</sup> h PAA where it reached its lowest value of 22% which is significantly ( $P < 0.05$ ) lower than the baseline value.

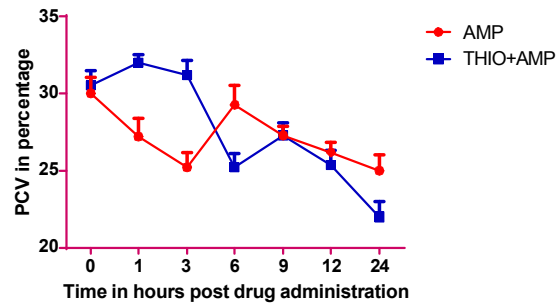


Fig. 1: Mean packed cell volume (PCV) before and following treatment of rabbits with both Ampicillin and Thiopentone sodium anaesthesia. Administration of ampicillin alone at the dose of 10mg/kg body weight was observed to induce significant decline in PCV while the combination of both drugs (thiopentone sodium at 20mg/kg body weight and ampicillin as above) further induce more significant decrease in PCV values which do not return to baseline values with the 24 hrs of the study. All data are presented as mean  $\pm$  SD.

**Mean Haemoglobin Concentration:** The mean Hb concentration for the first and second experiment is shown in Fig. 2. In the first experiment, administration of ampicillin alone at the dose of 10 mg/kg body weight was observed to induce consistent decrease in Hb concentration from the baseline value of 11.9 g/dl to 13.5 g/dl at the 6<sup>th</sup> h PAA. The Hb concentration was decreased from the 9<sup>th</sup> h through the 24<sup>th</sup> h PAA, reaching a value of 9.8 g/dl. However, in the second experiment, when ampicillin was administered following recovery from thiopentone sodium anaesthesia (at 20 mg/kg body weight), Hb concentration was observed to decline more than when ampicillin was administered alone. The Hb value was declined from the baseline value of 11.8 to 8.1 g/dl at the 6<sup>th</sup> h PAA. Thereafter, it was increased shortly at the 9<sup>th</sup> h and steadily declined again to 8.2 g/dl through the 24<sup>th</sup> h PAA.

**White Blood Cell (WBC) Count:** The mean WBC count of the rabbits before and following treatments are shown in Fig. 3. The administration of ampicillin alone at the dose of 10 mg/kg body weight in the first experiment was observed to induce a significant ( $P < 0.05$ ) decrease in the WBC count at the 3<sup>rd</sup> h PAA from the baseline value of  $146 \times 10^9/L$  to  $88 \times 10^9/L$ . However, the value increased to  $146 \times 10^9/L$  again at the 6<sup>th</sup> h and then declined again through the 24<sup>th</sup> h to a value of  $109 \times 10^9/L$  which is significantly ( $P < 0.05$ ) lower than the baseline value.

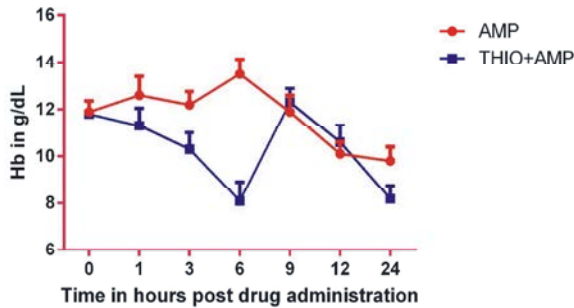


Fig. 2: Mean Haemoglobin (Hb) concentration before and following treatment of rabbits with both Ampicillin and Thiopentone sodium anaesthesia. While administration of ampicillin alone at the dose of 10mg/kg body weight did not induced significant decline in Hb, the combination of both drugs (thiopentone sodium at 20mg/kg body weight and ampicillin as above) induce significant decrease in Hb values especially at the 6<sup>th</sup> h and this decrease did not return to baseline values within the 24 h of the study. All data are presented as mean  $\pm$  SD

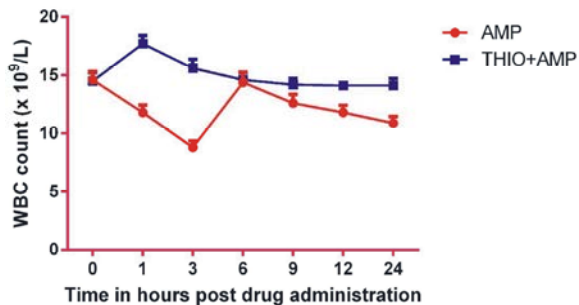


Fig. 3: Mean white blood cell (WBC) count before and following treatment of rabbits with both Ampicillin and Thiopentone sodium anaesthesia. While administration of ampicillin alone at the dose of 10mg/kg body weight was observed to induced significant decline in WBC especially at the third h post administration, the combination of both drugs (thiopentone sodium at 20mg/kg body weight and ampicillin as above) induce no significant alterations in WBC values and the values for both groups returned to baseline values starting from the 6<sup>th</sup> h post ampicillin administration. All data are presented as mean  $\pm$  SD.

In the second experiment, the WBC count of the rabbits when thiopentone sodium was administered (at 20 mg/kg body weight) prior to ampicillin administration was observed to decrease consistently from the baseline value

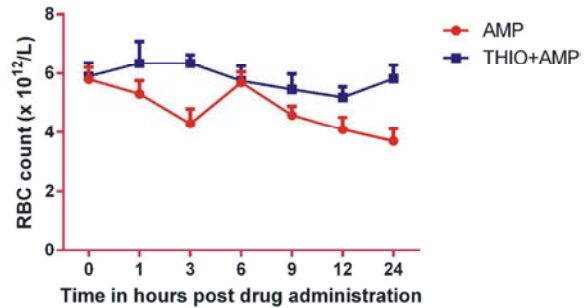


Fig. 4: Mean red blood cell (RBC) count before and following treatment of rabbits with both Ampicillin and Thiopentone sodium anaesthesia. The administration of ampicillin alone at the dose of 10mg/kg body weight was observed to induced a decline in RBC especially at the third h post administration which remained low even at the 24<sup>th</sup> h post administration while the combination of both drugs (thiopentone sodium at 20mg/kg body weight and ampicillin as above) induce no significant alterations in RBC values. All data are presented as mean  $\pm$  SD

of  $145 \times 10^9/L$  to a value of  $141 \times 10^9/L$  at the 24<sup>th</sup> h PAA which is not significantly different from the baseline value.

**Red Blood Cell (RBC) Count:** The mean RBC count for both the first and the second experiment is shown in Fig. 4. In the first experiment, administration of ampicillin alone at the dose of 10 mg/kg body weight was observed to induce a significant ( $P < 0.05$ ) decrease in RBC count from the baseline value of  $5.8 \times 10^{12}/L$  to  $4.3 \times 10^{12}/L$  at the 3<sup>rd</sup> h PAA. The RBC value increased again to  $5.75 \times 10^{12}/L$  at the 6<sup>th</sup> h PAA after which it then steadily declined through the 24<sup>th</sup> h to  $3.7 \times 10^{12}/L$  which is significantly ( $P < 0.05$ ) lower than the baseline value. However, in the second experiment during which thiopentone sodium was administered (at 20 mg/kg body weight) prior to ampicillin, the RBC count was decreased steadily and consistently from the baseline value of  $5.9 \times 10^{12}/L$  to  $5.2 \times 10^{12}/L$  at the 12<sup>th</sup> h PAA. The RBC count then increase slightly through the 24<sup>th</sup> h to  $5.82 \times 10^{12}/L$  which is not significantly different from the baseline value.

**Mean Corpuscular Volume (MCV):** The mean corpuscular volume (MCV) as determined in this study is presented in Fig. 5. The administration of ampicillin alone at the dose of 10mg/kg body weight was observed to induce increase in the MCV values from the baseline

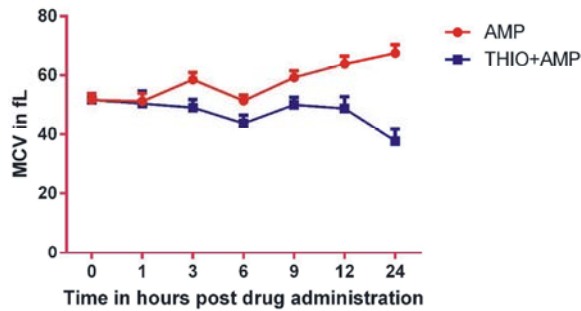


Fig. 5: Mean values for the Mean corpuscular volume (MCV) before and following treatment of rabbits with both Ampicillin and Thiopentone sodium anaesthesia. The administration of ampicillin alone at the dose of 10mg/kg body weight was observed to induced an increase in MCV especially at the 24<sup>th</sup> h post administration while the combination of both drugs (thiopentone sodium at 20mg/kg body weight and ampicillin as above) on the contrary, induce a decline in MCV values with the lowest value observed at the 24<sup>th</sup> h post drug administration. All data are presented as mean ± SD

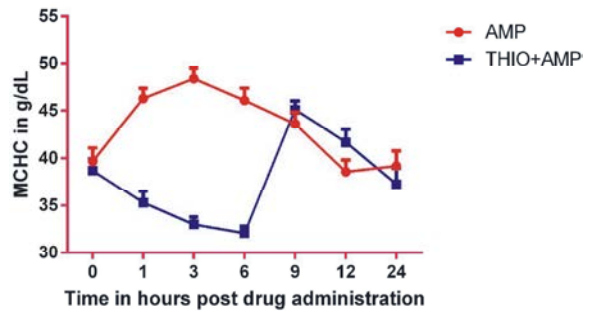


Fig. 7: Mean corpuscular haemoglobin concentration (MCHC) before and following treatment of rabbits with both Ampicillin and Thiopentone sodium anaesthesia. The administration of ampicillin alone at the dose of 10mg/kg body weight was observed to induce significant increase in MCHC values at the first and third hour post ampicillin administration. The combination of both drugs (thiopentone sodium at 20mg/kg body weight and ampicillin as above) induced a significant decline in MCHC values with the lowest value observed at the 6<sup>th</sup> h post drug administration. All data are presented as mean ± SD

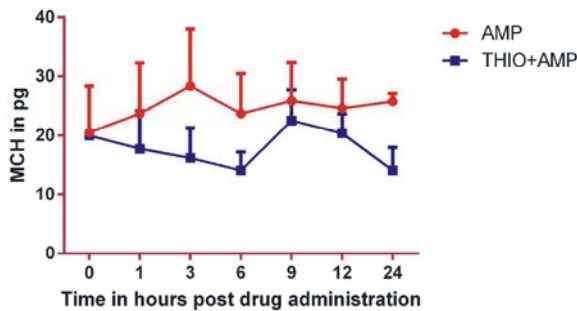


Fig. 6: Mean values of Mean corpuscular haemoglobin (MCH) before and following treatment of rabbits with both Ampicillin and Thiopentone sodium anaesthesia. The MCH value was not significantly altered following the administration of ampicillin alone at the dose of 10mg/kg body weight while the combination of both drugs (thiopentone sodium at 20mg/kg body weight and ampicillin as above) on the contrary, induce a significant decline in MCH values with the lowest value observed at the 6<sup>th</sup> and 24<sup>th</sup> h post drug administration. All data are presented as mean ± SD

In the second experiment, when thiopentone sodium was administered (at 20 mg/kg body weight) prior to ampicillin, the MCV values were declined from the baseline value of 51.7 fL to 43.8 fL at the 6<sup>th</sup> h and then subsequently, to 37.8 fL at the 24<sup>th</sup> h PAA. This value is significantly ( $P < 0.05$ ) lower than the baseline value.

**Mean Corpuscular Haemoglobin (MCH):** The mean values of the MCH as determined in this study is presented in Fig. 6. Administration of ampicillin alone at the dose of 10mg/kg body weight was observed to induce a transient increase from the baseline value of 20.5 pg to 28.4 pg at the 3<sup>rd</sup> h PAA. It eventually was declined from the 6<sup>th</sup> h through the 24 h where the value was 25.8 pg. The administration of thiopentone sodium (at 20 mg/kg body weight) prior to ampicillin was observed to induce a decline from the MCH baseline value of 20 pg to 14.1 pg at the 6<sup>th</sup> h PAA and it was increased to 22.5 pg at the 9<sup>th</sup> h before declining against to 14.1 pg at the 24<sup>th</sup> PAA.

**Mean Corpuscular Haemoglobin Concentration (MCHC):** The mean values of the MCHC as determined in this study are shown in Fig. 7. In the first experiment, administration of ampicillin alone at the dose of 10 mg/kg body weight was shown to induce a transient increase in MCHC value which eventually was declined to the

value of 51.7 fL to 58.6 fL at the 6<sup>th</sup> h and subsequently decreased to 51.4 at the 6<sup>th</sup> h PAA. The highest MCV value of 67.6 fL was observed at the 24<sup>th</sup> h PAA.

baseline value of 39.2 g/dl at the 24<sup>th</sup> h PAA. On the contrary, when thiopentone sodium was administered (at 20 mg/kg body weight) prior to ampicillin, MCHC values were decreased from the baseline value of 38.7 g/dl to 32.1 g/dl at the 6<sup>th</sup> h and later declined again to 37.3 g/dl at the 24<sup>th</sup> h PAA which is slightly lower than the baseline value.

## DISCUSSION

The application of appropriate antimicrobial prophylaxis can reduce the chances of postoperative wound infections as well as the cost of full antimicrobial regime. The secondary indications for antibiotic prophylaxis include clean operations in patients with compromised immunity or patients in whom the consequences of wound infections following surgery could be disastrous [2, 20]. The antibiotic, ampicillin chosen for this study is a semisynthetic penicillin which is often prescribed in clinical practice for both therapeutic and prophylactic purposes to reduce post-operative wound infection [18, 21, 22]. The decrease in PCV observed in this study following administration of ampicillin could be due to haemodilution. This finding is consistent with an earlier study reported by Al-Mayah and Al-Ahmed, [23] where the administration of ampicillin was observed to induce decrease in PCV values. However, the administration of thiopentone sodium prior to ampicillin was observed to induce a further decrease in PCV values beyond the values obtained when ampicillin was administered alone. The value failed to return to the baseline values 24 h post drug administration, implying that ampicillin's effect of PCV is beyond 24 h post drug administration. This appears to be the first study reporting synergistic effect of ampicillin and thiopentone sodium on PCV in rabbits. The further decrease induced by the thiopentone sodium could be due to splenic and capillary sequestration [24].

The use of simple and economic tests such as haematological parameters to rule out diseases of high clinical severity is appealing since it is effective and reduces healthcare costs [25]. The decrease in Hb values following ampicillin administration alone could be due to ampicillin induced haemodilution which is in accord with the findings reported by Al-Mayah and Al-Ahmed, [23] where ampicillin administration was reported to induce decline in Hb values. In the said study, the decrease in Hb value was ascribed to haemodilution. The administration of thiopentone sodium prior to ampicillin was observed to induce a significant decline in Hb values. This decline in

Hb value was observed to be significantly lower than the decline induced by ampicillin alone. Even though thiopentone sodium administration was reported to induce a non-significant decline in Hb values [26-28], in this study the administration of thiopentone sodium anaesthesia prior to ampicillin was observed to induce a significant decrease in Hb values, implying that the combined administration of both thiopentone sodium and ampicillin has greater or rather synergistic influence on Hb concentrations in rabbits and this needs to be taken into account when these drugs are to be administered concurrently in rabbits especially during surgery.

Investigating hematological changes enables the clinician to establish an effective therapeutic intervention that is early enough to prevent or mitigate the occurrence of major complications [29]. Administration of ampicillin at 10mg/kg body weight in the rabbits was observed to induce decline in WBC count. Information on how ampicillin induce decrease in WBC count appears to be scanty or rather limited. The available data are those from chicken [23] and owing to the difference in specie, caution must be taken in relating the findings. The lowest WBC was observed at the 3<sup>rd</sup> h PAA and we opined that the decrease was due to the ampicillin administration as this corresponded with peak plasma concentration of ampicillin [30]. However, administration of thiopentone sodium prior to ampicillin was observed to induce a less significant decrease in WBC count. This suggested that the combined administration of thiopentone sodium and ampicillin has a lesser effect on WBC count. This finding is in agreement with those obtained by Edjtehadi, Radi *et al.* [26, 28] who reported decline in WBC count following thiopentone sodium administration.

Haematological parameters could either provide or support an objective assessment of the health status and or diagnosis of different pathological conditions [31]. The decrease in RBC count observed in this study following ampicillin administration could be due to ampicillin-induced haemodilution. Although Studies reporting the effect of ampicillin RBC in rabbits are limited, an earlier study in chicken. Al-Mayah and Al-Ahmed, [23] reported relatively similar findings that ampicillin administration induce decline in RBC count. Similarly, the decrease in RBC count when thiopentone sodium was administered prior to ampicillin in this study is in agreement with an earlier study obtained by Saba and Oridupa, [27] who reported a decline in RBC count following thiopentone sodium anaesthesia in West African Hinged-back tortoise. Effect of thiopentone sodium and ampicillin on RBC in rabbit are scanty if

available, hence our findings on the effects of these drugs on RBC are discussed in the context of other related studies in other species.

The administration of ampicillin alone was observed to induce an increase in mean MCV values. However, the administration of thiopentone sodium prior to ampicillin was observed to induce a decrease in MCV value. This finding is in accordance with those reported by Saba and Oridupa, [27]. Significant decline in MCV value following administration of thiopentone sodium was reported. Even though MCH values were not significantly different from the baseline value in administration of ampicillin alone and combination of both, the administration of ampicillin alone was observed to induce a significantly higher value than the MCH values obtained following the combined administration of thiopentone and ampicillin. The combined administration of these 2 drugs may account for the difference in the findings of this study as against previous studies obtained by Al-Mayah and Al-Ahmed, Saba and Oridupa, [23, 27] who reported significant decrease in MCH values following administration of ampicillin and thiopentone sodium in chicken and tortoise respectively. The administration of ampicillin alone and in combination with thiopentone sodium did not induce any significant changes in the MCHC values. These findings are in accordance with the findings obtained by Al-Mayah and Al-Ahmed, Saba and Oridupa, [23, 27] who reported insignificant changes following either ampicillin or thiopentone sodium administration in separate experiments.

### CONCLUSION

The results obtained from this study has shown that administration of ampicillin has influence on haematological parameters of rabbits. This influence was observed to be higher when thiopentone sodium was administered prior to ampicillin and this appears to be the first report on the synergistic effect of ampicillin in combination with thiopentone sodium on haematological parameters in rabbits. In view of the wide gained acceptability of the rabbit as a laboratory animal model in different experimental procedures including surgery, the findings of this study is handy and invaluable. Based on the findings of this study, it can be concluded that administration of ampicillin following recovery from thiopentone sodium anaesthesia could exacerbate the effect of ampicillin on the haematological parameters of rabbits.

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