

*Full Length Research Paper*

# Effects of phenylalanine and glycine on some toxic effects of chloramphenicol

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We investigated the effects of concurrent administration of the amino acids, phenylalanine and glycine, on chloramphenicol (CAP)-induced haematotoxicity and histopathological effects in rats infected with *Klebsiella pneumoniae*. The study was carried out for 21 days in which haematological and histopathological changes were monitored in the infected animals treated with either of these amino acids or their combination and chloramphenicol. After 7 days of treatment, the amino acids suppressed the decreases recorded in anaemia-related haematological parameters. The order of potency is as follows: CAP/glycine > CAP / glycine / phenylalanine > CAP/phenylalanine > CAP alone > negative control. These decreases were significantly ( $P < 0.05$ ) different from the pretreatment values. Increases in the white blood corpuscle (WBC) count were recorded in all the groups. Phenylalanine and glycine reduced the proliferation of WBC, an indication of the ability to control progression of infections. The order for this activity is: CAP/phenylalanine/glycine > CAP/glycine > CAP/phenylalanine > CAP alone > control. With the exception of the group which received chloramphenicol alone, all other treatment groups exhibited increases or no changes in PCV, HB and RBC on day 21 relative to day 7 values. The WBC counts in all the animals in the treated groups were decreased, with CAP/glycine treated group being the most remarkable. Of all the combinations used in the study, only CAP/glycine was effective in protecting the liver against the toxic effects of CAP. No appreciable protection was noted in the spleen of any of the groups. Phenylalanine, glycine and their combination when given concomitantly significantly, reduced the anaemia and histopathological changes associated with chloramphenicol administration.

**Key words:** chloramphenicol toxicity, glycine, haematotoxicity, phenylalanine.

## INTRODUCTION

Chloramphenicol (CAP) is one of the older broad-spectrum antibiotics, which grew in popularity because of its high antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria, rickettsia, chlamydia, and mycoplasma. It is particularly useful in infections caused by *Salmonella typhi* and *Haemophilus influenzae* (Ruef and Blaser, 2000). It is a potent and potentially toxic antibiotic, which is mainly bacteriostatic

in action (Ruef and Blaser, 2000; BNF, 2006). Its toxic effects include haematological (Daun et al., 1978; Mary et al., 1990; Lancaster et al., 1998), immunological and hypersensitivity reactions (Liphshitz and loewenstein, 1991), optic neuropathy (Beyer, 1978; Murayama et al., 1973), and skin reactions (van Joost et al., 1986). The most important adverse effect of CAP is on the bone marrow and it is one of the most common drugs that cause pancytopenia (Wallarstein et al., 1969).

CAP continues to be widely used in many parts of the world despite its known haematotoxic and other adverse effects (Holt et al., 1998). Since CAP is still one of the cheapest antibiotics, it is still commonly used in developing countries where it is readily available and affordab-

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le than newer and more expensive antibiotics (Ruef and Blaser, 2000). Consequently, a study on possible therapeutic maneuvers aimed towards attenuating the toxicity of CAP is worthwhile.

Ingestion of phenylalanine has been shown to reverse one of the early toxic effects of CAP on human bone marrow, namely, the cytoplasmic vacuolization of erythrocytes (Ingal et al., 1965). However, it was reported that phenylalanine inhibits the antibacterial action of CAP *in vitro* (Woolley, 1950). A subsequent study however, indicated that administration of phenylalanine in a dose equivalent to that of CAP enhances the antibacterial effects of CAP while larger doses reduced the drug's antibacterial efficacy (Cockburn et al., 1965). With respect to glycine, slight synergistic effect has been reported in carbenicillin/glycine combination against *Pseudomonas aeruginosa* (Gerberick and Gastric, 1980). Glycine also leads to the production of drug susceptible *Escherichia coli* from drug resistant ones, thus eliminating drug resistant strain (Tomoeda et al., 1975). The amino acid is proposed to act by inhibiting the incorporation of L-alanine into uridine-diphospho-N-acetylmuramic acid (Hishimama, 1970).

Sequel to the above findings and the therapeutic value of CAP in specific indications especially in developing countries, we decided to study the effect of concurrent administration of phenylalanine (a hydrophobic aromatic ring amino acid) and glycine (a hydrophilic straight chain amino acid) on the haematotoxicity and histopathological profile of CAP in rats infected with virulent *Klebsiella pneumoniae*.

## MATERIALS AND METHODS

### Preparation of inoculum

Clinical isolate of *K. pneumoniae* was purified after Gram staining by streaking in sterile nutrient agar plate. Maintenance and cultivation of the microorganism were carried out by weekly subculturing on different nutrient agar slants. The agar slants were stored in a refrigerator at 4°C after 24 h incubation at 37°C. The organism was activated before use by successive subculturing in 10 mL sterile nutrient agar slant for 3 consecutive days. In each case, the inoculum was usually a 24-h-old culture of *K. pneumoniae*, standard inoculum size of  $0.5 \times 10^5$  cfu/ml (McFarland) was used for the tests.

### Animals and experimental design

Thirty albino rats of either sex (aged 5 weeks) obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were acclimatized for one week in our laboratory before the commencement of the experiment. They were fed with commercial growers mash (Guinea feed, Nigeria) and allowed unrestricted access to clean drinking water. The animals were randomly divided into five groups of six rats per group. Each rat was infected by intraperitoneal injection of 0.5 ml of the standard *K. pneumoniae*

culture and followed immediately by the intraperitoneal administration of the following:

- Group 1: 100 mg/kg of chloramphenicol (CAP) alone
- Group 2: 100 mg/kg of CAP plus 50 mg/kg of phenylalanine
- Group 3: 100 mg/kg of CAP plus 100 mg/kg of glycine
- Group 4: 100 mg/kg of CAP plus 50 mg/kg of phenylalanine plus 100 mg/kg of glycine
- Group 5: 5 ml/kg of normal saline (Negative control treatment).

Drug dilutions were made with sterile normal saline. The animals were observed daily for progression of infection and any overt sign of toxicity. Animals that died were autopsied and examined for histopathological changes. On the 21<sup>st</sup> day, the survivors were sacrificed and again monitored histopathologically.

### Haematological studies

Blood was collected before commencement of treatment (day zero) and on the 7<sup>th</sup> and 21<sup>st</sup> days post commencement of treatment respectively. The blood, collected by tail snipping into plastic tubes containing ethylenediaminetetraacetic acid (0.75 mg/ml) were used for the haematological analysis. The packed cell volume (microhaematocrit method), total red blood cell count, total and differential white blood corpuscle count (haemocytometer method) and haemoglobin concentration were determined according to standard methods described earlier (Brain, 1994; Cole, 1980).

### Histopathological studies

The surviving animals were sacrificed on the 21<sup>st</sup> day. Slices of spleen and liver were fixed in 10% normal saline for 24 h. Subsequently, the histopathological examinations were carried out as described by (Leeson and Leeson, 1970)

### Statistical analysis

Results were expressed as mean  $\pm$  standard error of mean and analysed using Students' t-test. Differences in paired mean observations were regarded as significant at  $P < 0.05$ .

## RESULTS

Three of the five animals in group 5 (infected but given normal saline only) died before the 7<sup>th</sup> day while the remaining two died before the end of the experiment. Sclerosis of the tail region was also observed in this group. The following pattern of deaths was recorded in the respective group: group 1 (2 died), group 2 (2 died), group 3 (1 died), and group 4 (2 died). The haematological indices (pretreatment and 7 days post-treatment) are shown in Table 1 and 2 respectively. Haematological indices on the 7<sup>th</sup> day indicated that there were decreases in packed cell volume (PCV), haemoglobin (HB) and red blood cell (RBC) counts and some of these values were statistically different from the pretreatment values ( $P < 0.05$ ). With the exception of group 5 (negative control) indexes, the group given

**Table 1.** Pretreatment (baseline) haematological data.

Treatment	PCV (%)	HB (g/100ml)	WBC total count (x 10 <sup>3</sup> cells/ml)	RBC total count(x 10 <sup>6</sup> )	Neutrophils (%)	Lymphocytes (%)
Chloramphenicol	51.7 ± 7.66	17.2 ± 2.55	5.76 ± 6.6	5.5 ± 0.8.0	60.0 ± 5.56	38.3 ± 3.0
Chloramphenicol + Phenylalanine	45.3 ± 7.75	15.1 ± 2.53	6.96 ± 8.5	4.8 ± 0.82	67.7 ± 3.06	31.6 ± 2.85
Chloramphenicol + Glycine	42.0 ± 11.35	13.9 ± 3.79	6.96 ± 1.55	4.6 ± 1.6	55.0 ± 7.3	42.6 ± 8.0
Chloramphenicol +Phenylalanine + Glycine	45.0 ± 15.0	15.0 ± 5.06	6.20 ± 1.72	4.8 ± 1.6	58.0 ± 16.64	41.6 ± 16.0
Normal saline	47.6 ± 2.50	15.8 ± 0.81	6.20 ± 1.0	5.1 ± 0.3	61.3 ± 4.06	37.0 ± 2.65

**Table 2.** Effects of concurrent administration of the amino acids, phenylalanine and glycine, on chloramphenicol (CAP)-induced haematotoxicity in rats infected with *Klebsiella pneumonia* on the 7<sup>th</sup> day.

Treatment	PCV (%)	HB (g/100 ml)	WBC total count (x 10 <sup>3</sup> )	RBC count (x 10 <sup>6</sup> )	Neutrophils (%)	Lymphocytes (%)
Chloramphenicol	33.60 ± 5.51 <sup>*</sup> (-35.00)	11.30 ± 1.28 <sup>*</sup> (-34.30)	8.5 ± 1.14 <sup>*</sup> (+47.57)	3.5 ± 0.6 <sup>*</sup> (-36.36)	33.3 ± 5.04 <sup>*</sup> (-44.50)	66.7 ± 5.04 <sup>*</sup> (+105.70)
Chloramphenicol + Phenylalanine	37.3 ± 9.07 <sup>*</sup> (-17.66)	12.43 ± 3.03 (-17.68)	7.6 ± 1.4 <sup>*</sup> (+9.57)	4.2 ± 0.95 <sup>*</sup> (-12.50)	35.0 ± 1.0 <sup>*</sup> (-48.30)	65.0 ± 3.0 <sup>*</sup> (+74.15)
Chloramphenicol + Glycine	39.2 ± 9.74 (6.67)	13.05 ± 3.24 (-6.12)	7.36 ± 1.59 (+9.63)	4.1 ± 0.99 (-10.87)	44.8 ± 9.84 (-18.55)	54.7 ± 9.28 (+28.40)
Chloramphenicol +Phenylalanine + Glycine	40.66 ± 4.62 (-9.64)	13.5 ± 1.56 (-10.00)	6.3 ± 2.5 (+1.61)	4.35 ± 0.45 (-9.38)	29.6 ± 4.53 <sup>*</sup> (-48.97)	70.0 ± 5.0 <sup>*</sup> (+68.26)
Normal saline	24.0 ± 5.65 <sup>*</sup> (-41.46)	7.95 ± 1.90 <sup>*</sup> (-41.97)	17.9 ± 2.9 <sup>*</sup> (+155.00)	2.5 ± 0.36 <sup>*</sup> (-48.98)	34.0 ± 2.50 <sup>*</sup> (-44.54)	66.0 ± 2.82 <sup>*</sup> (+79.73)

\*Significant P < 0.05; values in parenthesis represents percentage decrease (-) or increase (+) in respective indices.

chloramphenicol (CAP) alone was the worst affected while the least affected was the group that received CAP plus glycine (Table 2). In contrast, increases in white blood corpuscle (WBC) count were noted in all the groups and the smallest increase was seen in CAP / phenylalanine/glycine group. The order of increase in WBC count is as follows: group 4 < group 3 < group 2 < group 1 < group 5 (Table 2). Lymphocytosis and neutropenia were quite evident on the day 7. The RBCs appeared hypochromic.

The result of haematological indices on day 21 is shown in Table 3. With few exceptions, there was a general increase in PCV, HB and RBC counts on day 21 relative to day 7 (Table 3). There was a decrease in WBC count in all the groups. (Table 3) The effects of the various treatments on development of anaemia was evaluated using mean changes in PCV, HB and RBC (Table 4) while the effects of treatments on remission of

infection was evaluated using mean changes in WBC count (Table 5)

The histopathological changes observed in the liver and spleen sections of rats 21 days after treatment are shown in Figures 1-5. Rats in group 1 (treated with CAP alone) had histological changes characterized by degeneration and necrotic changes in the liver. The sections were moderately to severely hyperaemic and with widespread haemorrhages. There was moderate phagocytosis of erythrocytes of Kupffers cells, while the sinusoids were moderately to severely distorted. The hepatocytes had cytoplasm that were either granular or vacuolated while the nuclei were pyknotic or at the stage of karyorrhexis. These changes were most severe in the portal areas.

Spleen sections from rats in the same group (group 1) had congested red pulp with moderate to severe phagocytosis of erythrocytes of splenic macrophages with mo-

**Table 3.** Effects of concurrent administration of the amino acids, phenylalanine and glycine, on chloramphenicol (CAP)-induced haematotoxicity in rats infected with *Klebsiella pneumonia* on the 21<sup>st</sup> day.

Treatment	PCV (%)	HB (g/100ml)	WBC total count	RBC total count	Neutrophils (%)	Lymphocytes (%)
Chloramphenicol	35.30 ± 3.21 <sup>*</sup> (-5.36)	11.8 ± 1.08 <sup>*</sup> (-5.07)	4.0 × 10 <sup>3</sup> ± 1.8 × 10 <sup>3*</sup> (-22.89)*	3.7 × 10 <sup>6</sup> ± 3.21 × 10 <sup>5*</sup> (-10.23)	32.0 ± 2.51	67.0 ± 2.5
Chloramphenicol + Phenylalanine	44.16 ± 1.13 <sup>*</sup> (+31.43)*	14.6 ± 3.74 (+29.20)*	8.3 × 10 <sup>3</sup> ± 2.08 × 10 <sup>3</sup> (-2.35)	4.7 × 10 <sup>6</sup> ± 1.2 × 10 <sup>6</sup> (+34.24)*	27.0 ± 8.54	69.6 ± 10.8
Chloramphenicol + Glycine	39.25 ± 11.16 (Negligible)	13.06 ± 3.24 (Negligible)	4.75 × 10 <sup>3</sup> ± 4.9 × 10 <sup>2*</sup> (-37.75)*	4.1 × 10 <sup>6</sup> ± 8.2 × 10 <sup>5</sup> (Negligible)	33.3 ± 2.8	66.0 ± 21.8
Chloramphenicol + Phenylalanine + Glycine	42.3 ± 7.63 (+4.03)	14.1 ± 2.55 (+4.44)	6.0 × 10 <sup>3</sup> ± 2.6 × 10 <sup>3</sup> (-4.76)	4.2 × 10 <sup>6</sup> ± 8.2 × 10 <sup>3</sup> (+3.91)	42.6 ± 5.85	57.0 ± 5.5 <sup>*</sup>

\*Significant P < 0.05; percentage increases (+) in PCV, HB and RBC values (index for amelioration of anaemia) and decreases (-) in WBC count (index for remission of infection) on the 21<sup>st</sup> day relative to the 7<sup>th</sup> day.

**Table 4.** Chloramphenicol (CAP)-induced mean percentage change in PCV, HB and RBC in rats infected with *Klebsiella pneumonia* with concurrent administration of the amino acids, phenylalanine and glycine: an index of the animals' ability to control progression of anaemia.

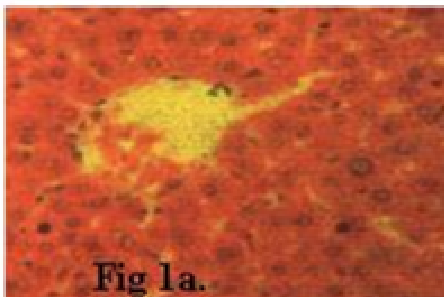
Treatment	Mean % change in PCV, HB, RBC values		Remarks
	% Decrease on the 7 <sup>th</sup> day	% Increase on the 21 <sup>st</sup> day	
Chloramphenicol	35.22	-6.87	Not potent. The drug did not reduce the animals' susceptibility to anaemia on the 7 <sup>th</sup> day, and the condition did not improve on the 21 <sup>th</sup> day.
Chloramphenicol + Phenylalanine	15.95	31.64	Potent with slow action. The efficacy was low on the 7 <sup>th</sup> day but very potent on the 21 <sup>st</sup> day, indicating slow onset but with prolonged duration of activity.
Chloramphenicol + Glycine	7.89	Negligible	Potent with steady action. The combination had fast onset of action, which peaked on the 7 <sup>th</sup> day and subsequently maintained a steady of activity.
Chloramphenicol + Phenylalanine + Glycine	9.71	4.12	Potent with continued action. Similar effect as above but with lesser efficacy.

derate to severe haemosiderin deposition. The splenic follicles were moderately to severely reactive. In group 2 (CAP plus phenylalanine), the histological changes in liver sections were similar to those in group 1. The circulatory disturbances observed were degenerative with moderate to severe haemosiderosis; the necrotic changes were as described for rats in group 1. Spleen sections collected from rats in the same group 2 had congestion of the red pulp and hypoplasia of the white pulp as described for the group 1 rats. In group 3 rats, the circulatory disturbances in the liver were less severe. In addition, the hepatocytes had fairly normal morphology

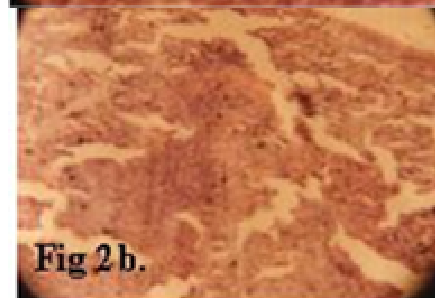
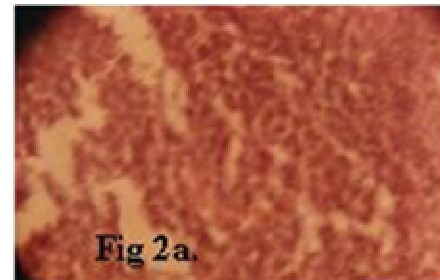
(cytoplasm and nucleus). Spleen section equally showed severe congestion of red pulp and highly reactive splenic follicles. In group 4 rats (CAP plus phenylalanine plus glycine), liver sections also had wide spread areas of hepatocytes degeneration and necrosis with the circulatory disturbance comparable to that reported for group 1 rats. There was moderate to severe distortion of the lobular architecture. The liver and spleen sections were congested with moderate to severe haemosiderosis, while the splenic follicle showed severe hyperplasia similar to these reported for rats in group 1.

**Table 5.** Chloramphenicol (CAP)-induced mean percentage change in WBC in rats infected with *Klebsiella pneumoniae* with concurrent administration of the amino acids, phenylalanine and glycine: an index of the animals' ability to control progression of the infection.

Treatment	Mean % Increase in WBC on the 7 <sup>th</sup> day	Mean % Decrease in WBC on the 21 <sup>st</sup> day	Remarks
Chloramphenicol	31.94	2.35	Potent with slow action. The drug had the least control of infection initially but reduced the infection with very high potency later.
Chloramphenicol + Phenylalanine	22.13	47.37	Least potent with little or no action. The combination had less effect over the infection at the initial stage with corresponding least ability to reduce increase in WBC count on the 21 <sup>st</sup> day.
Chloramphenicol + Glycine	9.63	37.75	Potent with fast action that persists. The drugs were less effective in the control of infection at the initial stage and with time, the efficacy increased resulting in 37.75% decrease in WBC on the 21 <sup>st</sup> day.
Chloramphenicol + Phenylalanine + Glycine	1.61	4.76	Potent with fast action that continues slowly. The combination was the most effective in controlling infection at the initial stage as seen with a very little increase in WBC count of 1.6 % and continues slowly to reduce the infection.



**Figure 1.** 1a. Normal hepatocytes (control). 1b. Normal spleen (control).

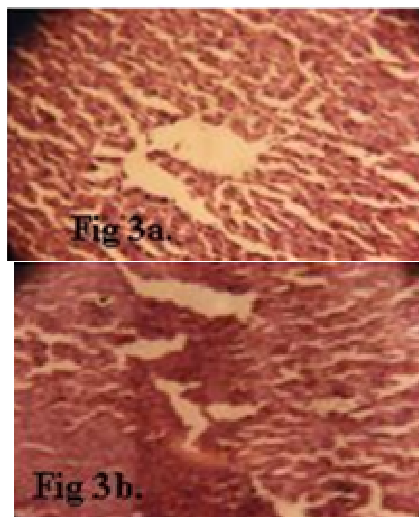


**Figure 2.** Chloramphenicol alone. 2a. Liver showing degeneration and necrotic changes induced by chloramphenicol. 2b. Spleen showing congested red pulp with moderate to severe phagocytosis of erythrocytes by splenic macrophages with moderate to severe haemosiderosis.

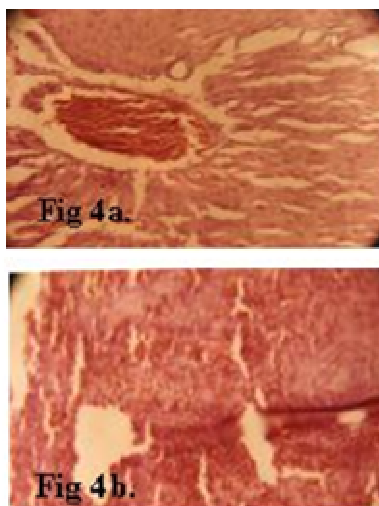
## DISCUSSION

The progressive decrease in PCV, RBC and HB counts noted in the group treated with chloramphenicol alone when compared with the other test groups supports the fact that chloramphenicol (CAP) induces anaemia as one

of its most common toxic effect (Holt and Bajoria, 1999). Red Blood Cell (RBC), Packed Cell Volume (PCV) and haemoglobin (HB) measurements are necessary in the assessment of anaemia [Odotola, 1992]. On day 7, CAP/

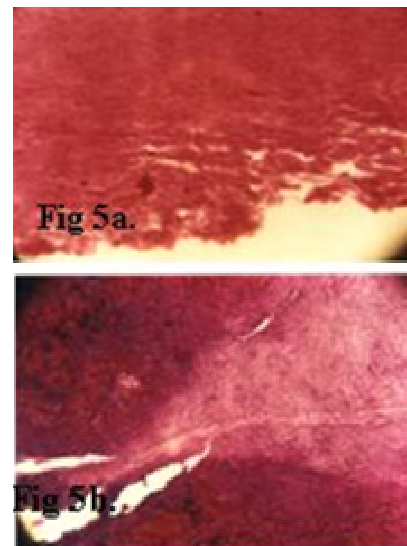


**Figure 3.** Chloramphenicol + phenylalanine. 3a. Liver with widespread area of hepatocytes degeneration and necrosis as in Figure 2 above. 3b. Spleen similar as in Figure 2 above.



**Figure 4.** Chloramphenicol and glycine. 4a. Liver showing cytoplasm and nucleus (normal morphology). 4b. Spleen indicating congested red pulp and highly reactive splenic follicle.

glycine combination produced the least decrease in RBC, PCV and HB counts, followed closely by CAP/phenylalanine/glycine combination. This indicates a fast onset of action with respect to ability to ameliorate progression of anaemia induced by the infection and/or CAP administration. CAP/glycine concurrent administration produced negligible percentage difference in the above red cell indices on day 21 when compared with the values obtained on day 7. This shows that the capacity of the combi-



**Figure 5.** Chloramphenicol+ phenylalanine + glycine. 5a. Liver similar to the observation in Figure 3. 5b. Spleen congested with moderate to severe haemosiderosis, splenic follicle shows severe hyperplasia.

nation to attenuate progression of anaemia peaked on day 7 and remained constant till day 21. Conversely, CAP/phenylalanine combination, which displayed significant decrease in RBC, PCV and HB counts on day 7, exhibited the highest percentage increase in the above haematological indices, signaling slow onset but persistent increase in activity. This correlates with earlier study that phenylalanine reverses the toxic effects of CAP on bone marrow (Ingal et al, 1965).

There is proliferation of lymphocytes (mature WBC) during infection by microorganisms (Kanfmann, 1988). Hence, the ability of CAP/phenylalanine/glycine combination to produce the least percentage increase in WBC count may correlate with potency to control infection by the *K. pneumonia*. This potency was progressively maintained and on day 21, the value was almost similar to the pretreatment value. CAP/glycine combination exhibited similar trend, with WBC count on day 21 even smaller than the pretreatment value (not significant,  $P < 0.05$ ). These findings tally with earlier one which demonstrated that glycine displays synergistic activity when combined with some antibiotics (Gerberick and Gastric, 1980). Better control of infection will lead to improvement in haematological indices. The ability of CAP/glycine combination to control proliferation of WBC (an indication of infection) may explain the ability of the combination to check decreases in RBC, PCV and HB counts with the best potency among the treated groups.

Histopathological study indicated that only the CAP / glycine combination protected the liver against the toxic effects of CAP. Other combinations did not confer appre-

ciable protection against CAP-induced toxicity in any of the body organs tested.

The results of our study revealed that co-administration of CAP with glycine or phenylalanine or both attenuated some toxic effects of CAP. The protection was most pronounced on the haematological untoward effects. Concurrent administration of the three agents produced the least change in WBC count, an indication of best potency to control progression of the infection. CAP/glycine or CAP/phenylalanine combination checked the decreases in RBC, PCV and HB counts in the CAP treated groups but CAP/glycine combination appear to be better; indicating superior ability to control anaemia induced CAP and or the infection. Although both CAP/phenylalanine/glycine and CAP/glycine combinations showed appreciable ability to control both the progression of anaemia and infection in the treated animals, the later combination, based on general performance assessment, seems to be better in protecting against the toxic effects.

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