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EFFICACY OF CLINDAMYCIN, YEAST *(SACCHAROMYCES CEREVISIAE*) AND CLINDAMYCIN-*SACCHAROMYCES CEREVISIAE* COMBINATION VERSUS TOLTRAZURIL ON EXPERIMENTALLY INDUCED COCCIDIOSIS IN LAMBS

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ABSTRACT

Coccidiosis is an economic important disease among sheep that results in a great adverse effect on their health condition. The present study aimed to compare the efficacy of clindamycin, Yeast (*Saccharomyces cerevisiae*), clindamycin- *Saccharomyces cerevisiae* combination and toltrazuril on experimentally infected lambs with a mixed infection of *Eimeria ovinoidalis* and *E. crandallis* oocysts. The following up of the drugs' efficacy was done by assessing the fecal oocyst count on the first day of oocysts shedding (12 day post infection, dpi) and on 13th, 15th, 19th, 22nd and 26th dpi with observation of the day of disappearance of the clinical symptoms. Ruimnal protozoa count and viability were estimated on 12th, 19th and 26th dpi to detect the effect of the oral administration of the used drugs on rumen viability. Also the blood parameters were measured on the same days to assess the influence of *Eimeria* infection on the haematological value and consequently the efficacy of the applied treatment on them. In the present study both toltrazuril and clindamycin- *Saccharomyces cerevisiae* combination could significantly ($P< 0.05$) reduce the fecal oocyst count with disappearance of the clinical symptoms by 26th dpi and they could significantly improve the values of the blood parameters of the infected animals. The later mentioned drug exhibited a significant increase in the ruminal protozoal count and maintain viability, while toltrazuril showed a significant reduction of both of them.

KEYWORDS: Clindamycin, *Eimeria, Saccharomyces cerevisiae*, Sheep, Toltrazuril

INTRODUCTION

Sheep coccidiosis is caused by *Eimeria* species which is considered very important especially in pre-weaned and recently weaned lambs. Clinical coccidiosis results in even higher financial losses for producers because of medical treatment costs, a more severe effect on growth performance and sometimes death impact on lambs less than 3 months old, causing severe damage to the intestinal tract, diarrhea, dehydration, impaired weight gain, or weight loss and death in some cases (Helle 1970; Gjerde and Helle 1991; Reeg et al. 2005). Many treatments have been recommended for coccidiosis, of them lasalocid in lamb and calves (Foreyt et al. 1979) and sulfonamides in calves and lambs (Radostits et al. 2007), monensin in cattle, lambs and goats, decoquinate in calves and goat kids (Parker et al. 1986; Radostits et al. 2007), toltrazuril in lamb (Ghanem et al. 2008; Rodostitis et al. 2007) and amprolium (Ghanem et al. 2008) are effective. It was proved that clindamycin has an effect on some protozoan parasites as Malaria (Griffith et al. 2007; Lell and Kremsner 2002),Toxoplasma (Jeddi et al. 1997; Pleyer et al. 2007) and Babesia (Homer et al. 2000) where it interrupt the target protein synthesis in the apicoplast (Guay 2007). Clindamycin effect on Eimeria infection had been previously studied in rodent and goat (Yunus et al. 2005; Temizel et al. 2011 respectively). Moreover, the use of direct fed microbials

(DFMs), yeast or probiotics as alternatives for prevention of coccidiosis was proved to improve animal performance partly by maintaining a beneficial gut microflora (Callaway et al. 2008). Among yeast, *Saccharomyces cerevisiae* that has the GRAS status (Generally Recognized As Safe) from the US Food and Drug Administration and has demonstrated positive effects in different species such as broilers, calves, beef cattle, dairy cow, piglets, pigs, sows and rabbit (Auclair 2011) and it could improve the immunostatus and growth performance of coccidia-infected broilers (GAO et al. 2009). To our knowledge no information was available about the effect of clindamycin or *Saccharomyces cerevisiae* on coccidiosis in sheep. So the objectives of the present study were to determine the efficacy of clindamycin, yeast (*Saccharomyces cerevisiae*) and Clindamycin- *Saccharomyces cerevisiae* combination on experimentally infected lambs with oocysts of *Eimeria crandallis* and *E. ovinoidalis* in comparison to toltrazuril, through the assessment of fecal oocysts count, clinical symptoms, estimation hematological parameters of the treated and non treated animals as well as comparing the effect of these medicaments on ruminal protozoal count and viability of the treated animals.

MATERIALS AND METHODS

Collection and Prepration of the Inoculum

The fecal matter was collected from naturally infected sheep and it was examined for Eimeria oocysts by a coverglass flotation method using Sheather's sugar solution to concentrate the oocysts (Sloss and Kemp, 1978). The infected fecal material was mixed in 2.5 % potassium dichromate solution and was left for sporulation at room temperature for one week. The sporulated oocysts were subsequently quantified using the Mc master technique (Maff, 1986). A strict morphological criterion was used to specify Eimeria species present depending on the morphometric character according to Levine and Ivens (1986).

Experimental Animals

Thirty healthy lambs aged from 4-6 months, free from any infection, physically good and without a history of illness were used for experimental study. Those animals were rendered free from any parasitic infection and then were kept indoor in the farm of the faculty of veterinary medicine, Benha University under observation with daily examination of their feces to ensure they are free from any parasitic infection. They were fed on a balanced ration and fresh water was applied ad libidum. The experimental procedures were approved by the local committee of the Faculty of Veterinary Medicine, Benha University, Egypt and according to the guidelines of National Institute of Health (NIH) in Egypt. The animals were divided into six groups (group1–6) comprising of 5 animals each. Each animal in only five groups were infected with a single inoculum of an aqueous suspension containing 10^3 sporulated oocysts of essentially two pathogenic species of coccidia: *Eimeria crandallis* and *E. ovinoidalis* and the 6^{th} group was kept as a control negative.

The animals were kept under control with daily examination of their feces to determine the first day of oocysts shedding which was 12^{th} day post inoculation (dpi) and counting of the oocysts of each animal in each group in that day was done before the commencement of the treatment. On 13^{th} dpi, 1^{st}, 2^{nd} and 3^{rd} groups were administered orally for 14 days with clindamycin (Clindam, 300 mg Sigma pharmaceutical industries, Egypt) at a dosage of 10 mg/kg of body weight of animal/ day (according to Temizel et al., 2011), yeast (*Saccharomyces cerevisiae*) in ration 1kg /ton according to the manufacturer's guidelines (Moisnil,Saife VetMed Private Limited India) and Clindamycin- *Saccharomyces cerevisiae* combination respectively, while the 4^{th} group was provided by 2.5% solution of toltrazuril (Toltrasol 2.5%® Arab company for medical product, El-Abour City, industrial area, Egypt) twice with one week interval at a dosage rate of 20 mg/kg BW according to Pilarczyk et al. (1999), the 5^{th} and 6^{th} groups was kept as non treated control positive and control

negative groups respectively.

Collection and Processing of Rumen Liquor

The effect of the used drugs on rumen protozoa was estimated by evaluation the ruminal protozoal count and viability where rumen liquor from animals was collected with the help of a stomach tube and aspiration bottle on 12th, 19th and 26th dpi. One hundred ml of representative sample of rumen liquor was brought immediately to the laboratory in a closed container tightened with a rubber stopper to sustain anaerobic condition during transport. Samples of rumen liquor were sieved between two layers of gauze to remove debris. Two 5ml duplicate liquor were separately taken and diluted five times by saline solution and lugol's iodine to fix and stain protozoan cell. 0.1ml of the diluted ruminal fluid was poured on a clean glass slide which was then covered by a clean cover slide. The total protozoa count / 1ml = average count in 30 field X 1173 (area of the cover slide X 50). Each of the two duplicates was counted and the average was taken (Abou El-Naga 1967). Evaluation of the viability of the ruminal protozoa was done by counting the proportion of motile ciliates under microscope (Nsabimana et al. 2003).

Haematological Parameters

Blood samples were collected from all groups from the jugular vien in 10 ml sterilized tube containing EDTA (Benjamin 1984) at different interval during the experiment, on 12th, 19th and 26th dpi. The examined blood parameters were total leucocytic count (WBCs), lymphocytes count, monocytes count, granulocytes count, total erthrocytic count (RBCs), hemoglobin concentration (Hb), haematocrit concentration (HCT) and mean corpuscular hemoglobin concentration (MCHC). The reference normal level of the blood parameters of sheep was in accordance to Radostits et al. (2007).

Histopathology

Lamb that died during the experiment was autopsied and its intestine was fixed in 10% natural buffered formalin. Sections of paraffin-embedded tissue were stained with hematoxylin and eosin and examined under light microscope (Luna 1968).

Statistical Analysis

Data were analyzed by ANOVA followed by LSD multicomparison post- hoc test and Duncan post-hoc test using IBM SPSS Statistics 16 (SPSS Inc, U.S.). Differences between drugs were considered significant at $P< 0.05$.

RESULTS

Clinical Signs

The experimentally infected lambs *with E. crandallis* and *E. ovinoidalis* oocysts (figure 1; A & B) showed the clinical signs of coccidiosis on10th to13th dpi. Most of the clinical signs were more evident in the control positive group where they were suffering from diarrhea contains mucus or blood, tenesmus, loss of appetite, weakness and dehydration and one lamb on 22nd dpi, but the severity of the signs varied in the other treated group and subsides at different periods after the onset of treatment (Table 1).

Oocyst Counts

All the used drugs could significantly ($P< 0.05$) reduce the fecal oocyst count from 19th to 26th dpi compared to

12th dpi and control positive group, but they showed a significant increase in the counts compared to control negative group except toltrazuril and clindamycin- *Saccharomyces cerevisiae* combination which showed no significant difference (P> 0.05) in fecal oocyst count compared to control negative group on 26th dpi (167±33.33 and 247±23.33 respectively) (Table 2).

Ruminal Protozoa Counts and Viability

On 26th dpi, toltrazuril and clindamycin had a significant reduction (P< 0.05) on the ruminal protozoal count and viability compared to the other groups. Clidamycin-*Saccharomyces cerevisiae* combination showed a significant increase (P< 0.05) of the counts compared to control negative group with a slight significant reduction of the viability compared to 12th dpi, control positive and control negative groups, but this effect was significantly lower than that of toltrazuril and clindmycin groups. On the other hand *Saccharomyces cerevisiae* group exhibited a significant increase of the ruminal protozoal count(figure 1; C) with no effect on the viability on 19th and 26th dpi compared to 12th dpi, control positive and control negative groups (Table 3).

Haemtological Parameters

The median of the haematological parameters of each group were expressed on a box plot graph (1-8). The blood parameters of the control positive group was the most influenced where it showed increase in WBCs count, lymphocytes count and a significant decrease in RBCs count, Hb concentration, HCT and MCHC as compared to control negative group on 19th and 26th dpi. The drugs used in coccidiosis treatment showed a pronounced positive effect where they could improve the values of WBCs, RBCs, granulocytes counts, Hb concentration and HCT by 26th dpi compared to control positive group.

Histopathology

Only one lamb of the control positive group died on 22nd dpi, the examination of the histological section of its intestine showed the different developmental stages of *Eimeria* with hyperplasia in the epithelial cells of the intestine and infiltration with inflammatory cell represented by eosinophils (figure 2)

DISCUSSIONS

In the present study, the severe clinical symptoms of coccidiosis were associated with the massive invasion of the intestine with the second generation meront and gamont (Gregory et al. 1987) where clindamycin was able to overcome it and to significantly decrease the oocysts compared to control positive group and 12th dpi. The oocysts reduction effect of clindamycin may be due to its bacteriostatic effect as it inhibits the bacterial protein synthesis by inhibiting ribosomal translocation; this has an effect on repairing of the intestinal mucosa and preventing bacterial invasion that allow the animal to pass the effect of multiplying stages of *Eimeria* and relieving of symptoms. The inhibitory effect of clindamycin on aplicomplexan parasites was previously proved by Yunus et al. (2005) and Temizel et al. (2011) in mice and goat coccidiosis respectively, Plasmodium, Toxoplasma and Babesia (Lell and Kremsner, 2002).The reduction of ruminal protozoal count produced after clindamycin administration may be temporary as ruminal protozoa inhibition due to prolonged antibiotic feeding resulted in the selection of a resistant population in the rumen (Dennis et al. 1986) and the total number of protozoa apparently became adapted to the antibiotic within four weeks (Olumeyan et al.1986).

Saccharomyces cerevisiae could reduce the fecal oocyst count and to decline the clinical symptoms but it had the little effect compared to the other drugs.This inhibitory effect on protozoal adhesion was previously observed in case of *Entamoeba histolytica* trophozoites (Rigothier et al., 1994) which is produced by binding of the pathogens to the yeast cell wall, then induces a protective effect where complex *Saccharomyces cerevisiae*/ pathogen is rapidly eliminated from the digestive tract, also the competition between yeast and pathogens for binding to intestinal cells, since adhesion is crucial to the expression of the cytopathogenic effect (Gedek, 1989). While its great ability to significantly increase the ruminal protozoal countis attributed to increasing ruminal pH and consequently the numbers rumen protozoa (Doležal et al. 2011), this was also previously reported by Miranda et al. (1996).

Clindamycin- *Saccharomyces cerevisiae combination* showed no significant difference (P> 0.05) in fecal oocyst count compared to control negative group on 26th dpi, disappearance of the clinical symptoms on 22nd dpi and it raised the ruminal protozoal count. So the use of *Saccharomyces cerevisiae* along with clindamycin could restore the deleterious effect produced by the use of Clindamycin alone on the ruminal protozoa.

Upon comparing toltarazuril group with the other used drugs, it showed the most significant decrease of the fecal oocysts with disappearance of the symptoms in less time, but with a dramatic reduction of ruminal protozoal count and activity. This effect was previously recorded upon the use of many antiprotozoal compounds as dimetridazole and imidazole which proved to reduce ruminal protozoal activity (O'connor et al. 1970).

The alterations of the haemtological parameters recorded in the present study has been previously investigated in natural and experimental coccidiosis infections in sheep which include a reduction in erythrocytic count (RBCs) and hemoglobin (Hb) concentration (Rama et al. 1978; Hayat et al. 1990; Ghanem and Abd El-Raof 2005) but the used drugs could improve WBCs, RBCs, granulocytes counts, Hb concentaration and HCT by 26th dpi**.** No literatures were available about the effect of the aforementioned drugs on blood parameter**s**

CONCLUSIONS

It has been shown by this investigation that toltrazuril and clindamycin- *Saccharomyces cerevisiae* combination could significantly surpass the other used drugs as they could reduce the fecal oocyst count of *Eimeria* with no significant difference compared to control negative group on 26th dpi and to precede the other drugs in disappearance of the clinical symptoms. They also could bring the haemtological parameters close to their normal levels, but clindamycin-*Saccharomyces cerevisiae* combination has a beneficial effect on increasing of the ruminal protozoal countand maintaining their vitality as compared with toltrazuril treatment which has a substantial decrease of them.

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APPENDICES

Table 1: The Effect of the Used Drugs on Clinical Symptoms of *Eimeria* Infection

Group	Day (dpi) of Appearance of Clinical Symptoms	Clinical Symptoms	Day (dpi) by which the Clinical Symptoms Disappeared
C	10	intermittent diarrhea with blood and weakness, in appetence	25
S	12	Sever diarrhea and tenesmus	26
C/s	13	Diarrhea contain mucous and blood	22
T	10	Diarrhea, tenesmus and off food	20
Cp	13	Diarrhea contain mucous and blood , tenesmus, loss of appetite and dehydration	>26

Table 2: Mean Number of OPG in Sheep on 12^{th}, 13^{th}, 15^{th}, 19^{th}, 22^{nd} and 26^{th} Dpi among Different Groups Including: Clindamycin (C), Saccharomyces Cerevisiae (S), Clindamycin - Saccharomyces Cerevisiae Combination (C/S), Toltrazuril (T), Control Positive (CP) and Control Negative (CN) Groups

Groups	12^{th} dpi	13^{th} dpi	15^{th} dpi	19^{th} dpi	22^{nd} dpi	26^{th} dpi
C	$2250+144.33^{a}$	2233 ± 240.37^{a}	1503 ± 31.80^{b}	816 ± 60.09^{cd}	653 ± 54.87^{bc}	337 ± 18.56^{bc}
S	2496 ± 249.69^{a}	2474 ± 187.64^{a}	1820 ± 8819^{ab}	1200 ± 17.32^{bc}	877 ± 69.60^{b}	543 ± 72.19^{b}
C/S	2063 ± 219.87^{a}	2343 ± 29.62^{a}	1023 ± 14.52^{b}	610 ± 15.28^{d}	596 ± 3.33^{c}	247 ± 23.33^{bcd}
T	2423 ± 64.89^{a}	1996 ± 60.64^{a}	1620 ± 45.83^{b}	1327 ± 302.01^{b}	713 ± 36.67^{bc}	167 ± 33.33^{cd}
CP	2166 ± 13.332^{a}	2333 ± 202.67^{a}	2680 ± 90.19^{a}	3420 ± 215.95^{a}	10330 ± 156.95^{a}	12423 ± 212.63^{a}
CN	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{e}	0.00^{d}	0.00^{d}

abcd Differences between the values involving different letters in the same row were that are found to be statistically significant at $p < 0.05$.

Table 3: Mean Counting (10^5/Ml) and Viability of Ruminal Protozoa on 12th, 19th And 26th Dpi among Different Groups Including: Clindamycin (C), Saccharomyces Cerevisiae (S), Clindamycin- Saccharomyces Cerevisiae Combination (C/S), Toltrazuril (T), Control Positive(CP) and Control Negative Groups (CN)

Groups	Protozoal Counting			Protozoal Viability		
	12th Dpi	19th Dpi	26th Dpi	12th Dpi	19th Dpi	26th Dpi
C	4.20±0.22[a]	2.60±0.03[c]	1.8267±0.11[d]	88.64±1.60[a]	69.7133±0.60[b]	64.06±0.85[c]
S	3.23±0.11[b]	4.14±0.03[a]	4.1600±0.08[a]	87.85±0.33[a]	84.2767±0.50[a]	85.96±1.18[a]
C/ S	3.93± 0.86[ab]	3.23±0.15[b]	3.3100±0.06[b]	87.69±0.83[a]	83.1333±1.60[a]	81.60±0.58[b]
T	3.90±0.26[ab]	2.24±0.05[c]	0.5500±0.03[e]	89.28±0.16[a]	50.04±0.09[c]	48.80±1.76[d]
CP	3.50±0.05[ab]	3.36±0.87[b]	3.1700±0.03[bc]	86.20±1.78[a]	85.6300±2.42[a]	85.29±0.71[a]
CN	3.52±0.07[ab]	3.36±0.78[b]	3.03±0.0.3[c]	87.36 ±1.95[a]	87.2133±1.62[a]	88.47±1.60[a]

abcde Differences between the values involving different letters in the same row were that are found to be statistically significant at $p < 0.05$.

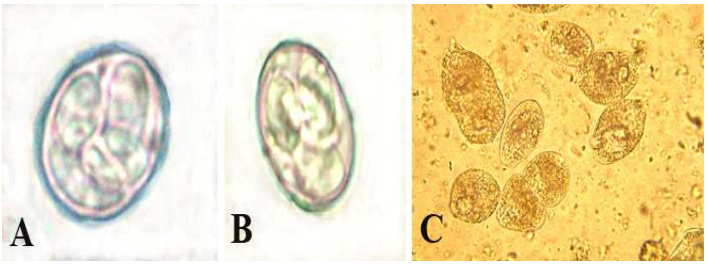


Figure 1: A. Eimeria Crandallis Sporulated Oocytst, B. Eimeria Ovinoidalis Sporulated Oocyst, C. Large Number of Ruminal Protozoa After Using of Saccharomyces Cerevisiae

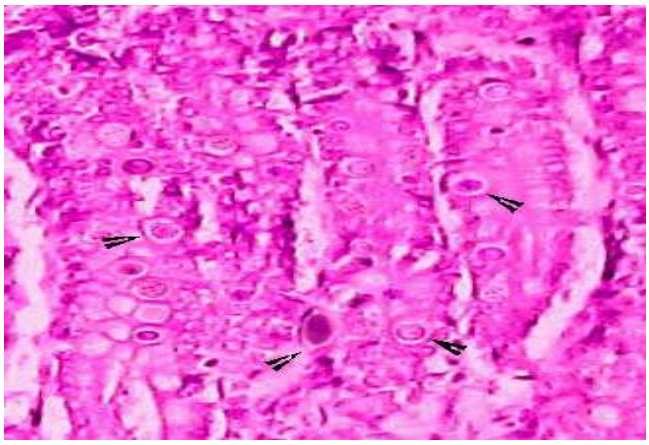
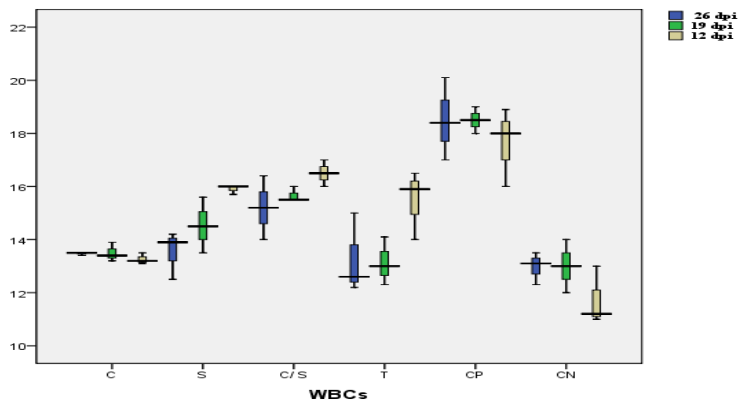
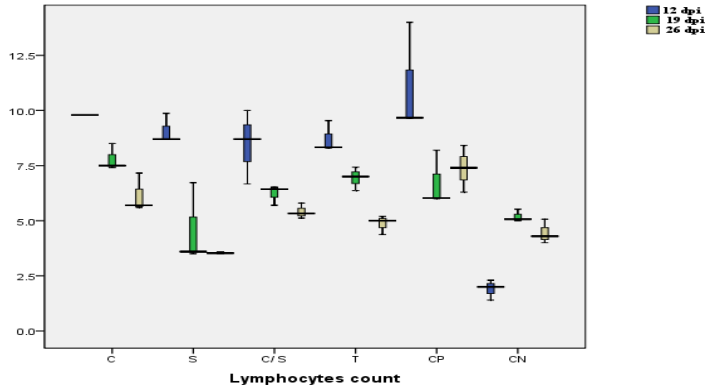


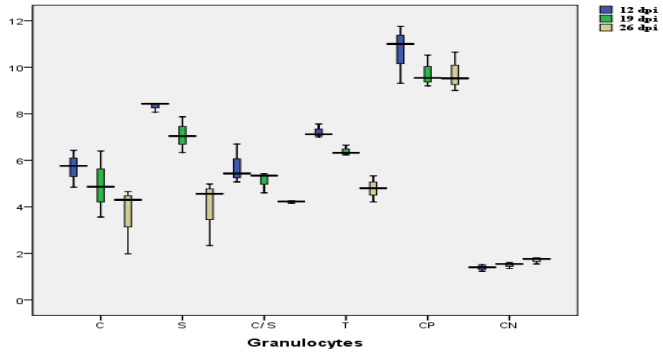
Figure 2: Developmental Stages of Eimeria in the Intestine of a Dead Lamb (Arrow Heads)



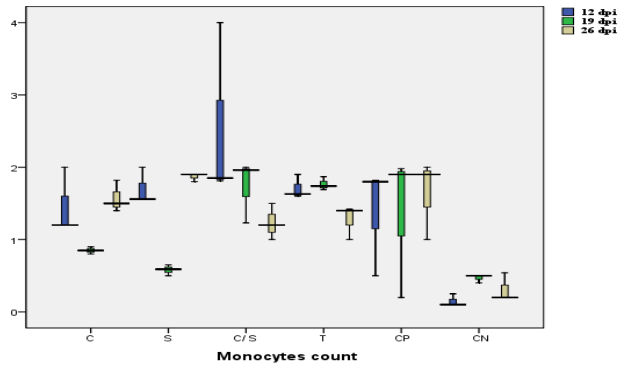
Graph 1



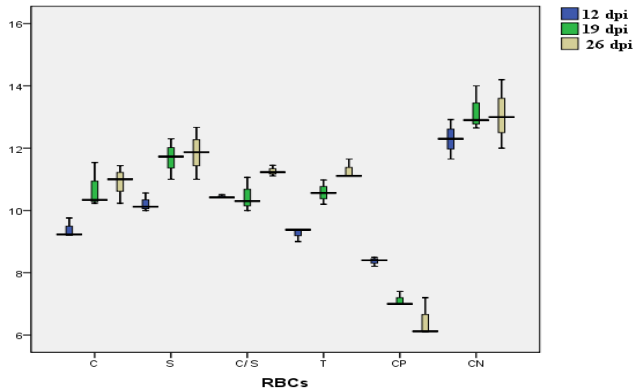
Graph 2



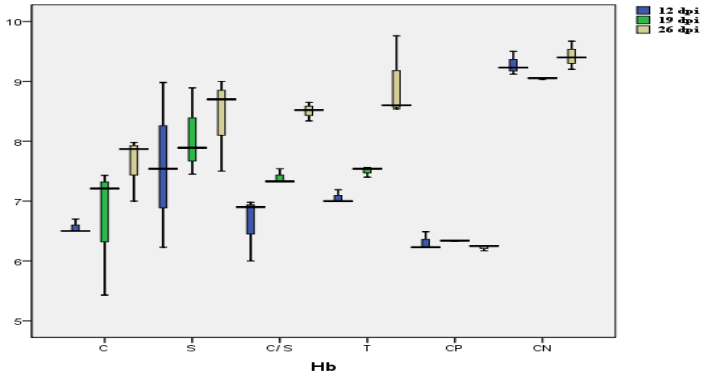
Graph 3



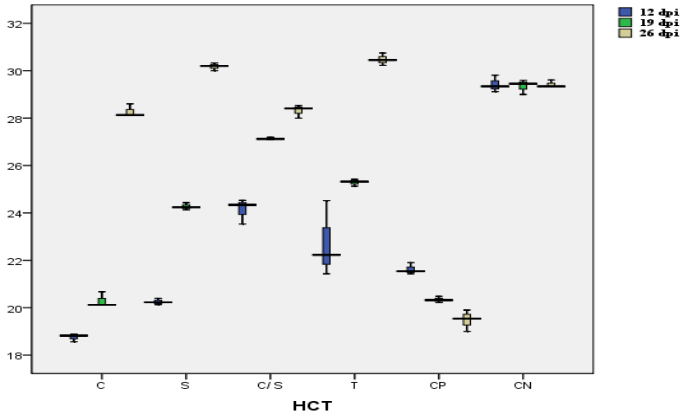
Graph 4



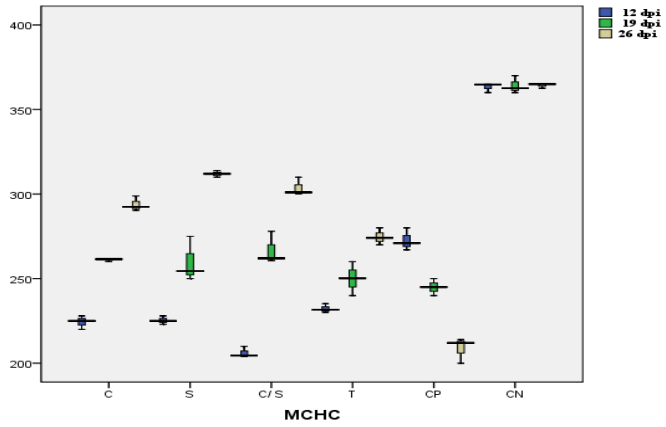
Graph 5



Graph 6



Graph 7



Graph 8

Graphs 1-8: Evaluation of Blood Parameters among Different Groups