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Research Paper

Effects of oxyclozanide (Tremacid®) preparation against fascioliasis on clinical and haematological parameters in cattle of Bangladesh

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Abstract

The effects of Oxyclozanide (Tremacid®) on some clinical (body weight) and haematological parameters (TEC, Hb, PCV, ESR and TLC) were determined in this study. Among 55 cattle, 10 were selected, which were suffering from fascioliasis and divided randomly into two groups. Each group was consisting of five cattle. One was treated with Tremacid® @ (15 mg/kg body weight). Other was kept as an infected control group. Before trials with Tremacid® initial body weight, EPG of liver fluke and hematological parameters were examined. During the experimental period the faecal samples, clinical and hematological parameters were examined on 7th, 14th, 21st and 28th days for the determination of effects of Tremacid®. A significant reduction of EPG count was found on 7th, 14th, 21st and 28th day of Tremacid® (61.87%, 71.22%, 76.98% and 84.53%) in a treated cattle group. The EPG of an untreated control group was significantly ($p<0.01$) increased about 3.97%, 7.64%, 11.04% and 64.89% respectively. Total TEC was increased after treatment with Tremacid® and decreased in an untreated control group significantly ($p<0.01$), Likewise, after treatment with Tremacid® Hb content and PCV were increased, and ESR was decreased. Total leukocyte count (TLC) was decreased and the body weight was increased after Tremacid® treatment in the group A. On the other hand, Hb content PCV and body weight was decreased significantly ($p<0.01$) in the group B.

Keywords: *Body Weight; Cattle; Fascioliasis; Haematological Parameter; Oxyclozanide.*

1. Introduction

Livestock production constitutes one of the principal means of achieving improved living standards in many regions of the developing world. Bangladesh is an agricultural based subtropical country. Livestock is considered to be the backbone of agriculture (Anonymous 1985). In Bangladesh, about 80 percent of the total population lives in rural areas. There are about 44.835 million ruminants (cattle, buffalo, goat, sheep 26.828, 0.544, 16.242, 1.221 million respectively) in Bangladesh (BBS 2010) which plays an important role in the rural economy (Kamaruddin 2003) and the livelihood of rural communities. It provides drought power, milk, and meat, input for crop production and soil fertility and raw material for industry. It is reported that more than 20% of the rural population of our country are engaged in this sub sector for their subsistence (Samad 1996). Livestock contribute approximately 11 percent of the animal protein requirements of humans. The main source of animal protein is livestock and their products. Cattle, buffalo, sheep and goats are the most important livestock in Bangladesh. About 90% of animal protein in our diet comes from fish and livestock. It contributes 2.67 percent to national GDP and 27.0 percent to agricultural GDP and 7 percent to the export earnings. Per capita income, is US$ 750, nearly half of the population (40 percent) live in absolute poverty, consume less than 2,122 calories per day and 19.5 percent are hard-core poor (Economic Index, 2010). Parasitism is one of the main constraints limiting livestock productions. It is a vulnerable condition for parasitic diseases in ruminants. Tropical climate together with the water logged and low-lying areas in Bangladesh favor the survival, multiplication, spread and perpetuation of animal parasites (Saiful et al 2003). *Fasciola gigantica*, which causes fascioliasis, infests 60 percent of ruminants in Bangladesh. Although this species is widespread in the country, its incidence is comparatively high in Sylhet, Chittagong, Chittagong Hill Tract (CHT), Dhaka, Netrakona, Barisal, Khulna and Faridpur districts. The known intermediate host in Bangladesh is the snail *Lymnaea auricularia*. Mortality of animals from parasitic diseases may not be alarming at times but their direct effects in terms of reduced milk, meat, wool, hide production, infertility and loss of stamina of working animals and especially zoonotic impact on human health are considerably greater (Baker and Muller, 1988).

There are so many important zoonotic parasitic diseases such as Hydatidosis, Fascioliasis, Settariasis, Trichinellosis, Ascariosis and Amphistomiasis, etc. (Schwabe, 1984). The importance of these diseases as a public health hazard, particularly in rural areas where a close association exists between man and domestic animal is well established (Kabir et al 2010). The Parasitic diseases are responsible for significant losses through morbidity and mortality in cattle in Bangladesh. Parasitism is the major cause hindering the development of livestock population in the country (Shahiduzzaman et al 1999). Several studies have indicated the incidence of different parasitic diseases and their seasonal prevalence in cattle of Bangladesh (Rahman, 1969, Rahman and Razzak, 1973). In cattle the prevalence of parasitic disease, especially fascioliasis was 30.37% (Kabir et al 2010). Fascioliasis is reported to be one

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of the important diseases of cattle and small ruminants in the country (Qadir 1981). It is wide spread in the country affecting 60 percent of the ruminants. They are the common anemia producing agents in indigenous cattle causing great economic losses to the farmers of Bangladesh. The mortality rates are 10% in sheep and goats and 5% in cattle and Buffaloes (BLRI, 2006). Among the various parasitic infections, fascioliasis is considered to be a major disease of ruminants in this country. Among them, fascioliasis occurred 60% in cattle, 99.99% in buffaloes, 12.29% in goats and 8.34% in sheep. It estimated an annual loss of Tk. 54.11 million due to fascioliasis in Bangladesh (Ghosh, 1988). 60% ineffectively of *Fasciola gigantica* in cattle at Dhaka and exclusively stressed that due to fascioliasis work-oxen needed to be replaced every second or third year (Kendall, 1954). Among all the problems hampering the livestock development, parasitic diseases occupy prime position in Bangladesh. The agro-ecological and geo-climatic condition of Bangladesh favors high prevalence of parasitic infestation. Parasitic diseases is also associated with anaemia and gastroenteritis (Soulsby, 1986) resulting loss of body weight, stunted growth, diarrhoea etc. that greatly hamper the normal growth and production of mortality, stunted growth, weight loss, decreased milk and meat production, draft power, market value of the animals, infertility and condemnation of carcasses during meat inspection. Science and technology are developing rapidly, but the national progress is not all the satisfactory due to national financial security. In Bangladesh, many drugs are being used for a long time to combat parasitic infection in livestock. The incidence of fascioliasis is mostly associated with low lying marshy and frequently inundated areas (Cockrill, 1974). In the absence of pasture dressing techniques, the affected animals have to be treated with anthelmintics. A large number of anthelmintics are now available in the market, which are being used by the field veterinarians and the quacks as well. Efficacy of anthelmintics is continuously constrained by many factors like under dosage, exclusive use of drugs of the same mode of action, substandard drugs and inappropriate use of anthelmintics. There is no anthelmintics use policy in the country as a result misuse and irritational administration is a widespreadpractice.

The losses due to liver fluke infection (fascioliasis) can be minimized by the prevention, control and treatment. There are no preventive measures taken in Bangladesh to control the parasitic disease. In developed countries, the principles of control of parasitic diseases are based on pasture and barn management and protective treatment (Rodistis et al 2000). But in Bangladesh, it is quite impossible because our farmer graze mixed animal in limited field and have lacked of knowledge on pasture land. So, we can prevent and control of parasitic diseases by using a routine prophylactic anthelmintic's measurement. A good number of effective anthelmintics are available in the market. Among these, Oxyclozanide (Tremacid®,Renata Ltd.), @ 15 mg/kg body weight) is wieldy used for the treatment of fascioliasis in domestic ruminants. This study aimed at evaluating the efficacy of Oxyclozanide against *Fasciola gigantica* in naturally infected cattle at a local dairy farm in Sreenagar Upazilla, Munshigonj district, Bangladesh. Under these circumstances, the present study on the fascioliasis in cattle under following objectives.

1) To determine the effects of Oxyclozanide (Tremacid®) on haematological parameters (TEC, Hb, ESR, TLC and PCV) in cattle.
2) To determine the effects of Oxyclozanide (Tremacid®) on a clinical parameter (body weight) in cattle.

2. Materials and methods

The experiment was conducted in Sreenagar milk shed area (Milk vita) in Munshigonj District, Bangladesh in collaboration with the department of Physiology and Pharmacology, Sylhet Agricultural University, Sylhet, Bangladesh for a period of 28 days to study the effects of Oxyclozanide (Tremacid®) against liver fluke infection (Fascioliasis) in cattle. In this study, the effects of Oxyclozanide (Tremacid®) on some hematological parameters and body weight were also determined. The research work was carried out from January, 2010 through June, 2010.

2.1. Experimental animals (cattle)

Among fifty five (55) cross bred ten (10) cattle of both sexes aged between 1.5 to 3 years were primarily selected in this study. All the cattle were examined for the presence of liver fluke (*Fasciola gigantica*).

2.2. The test drug

The bolus preparation of Oxyclozanide (Tremacid®,Renata Ltd., @ 15 mg/kg body weight) was selected for the experiment and purchased from Pharmaceutical store of local market. The drug was used for positive control and to compare the anthelmintics efficacy of Oxyclozanide (Tremacid®) in cattle.

2.3. Chemicals and reagents

a) Hydrochloric acid (0.14% HCl solution)
b) Saturated salt solution
c) Normal saline (0.9% Nacl solution)
d) Immersion of Iosan® (Novartis Bangladesh Ltd.)
e) Anti-coagulant (Sodium citrate 3.8%)
f) Hayem's solution

2.4. Instruments and appliances

a) Microscope
b) Pestle and mortar
c) Measuring balance
d) Handle and blade
e) Sterile cotton
f) F) Beaker
g) Haemacytometer
h) HelligeHaemometer
i) Hand gloves
j) Poly Bags
k) Tray

2.5. Selection of cattle

Among fifty five (55) cattle, ten (10) cattle were selected for this study that were suspected to suffer from liver fluke (*Fasciola gigantica*) infection (Direct smear faecal examination), and they were marked by tag at the neck. Detail clinical, physical and microscopic examinations of faecal sample for liver fluke egg counts by slide method were carried out on cattle over a week prior to commencement of treatment. All these cattle were maintained at the same altitude and under nearly identical conditions. They were kept in animals shed at night and part of the day. Wheat bran, rice polish, maize and salt are mixed together and supplied to the cattle up to 1-2 kg approximately daily. All the cattle were allowed for free pasture grazing for 2-3 hours daily. Plenty of water was also provided to all cattle.

2.6. Experimental design

Total 10 cattle infected by *Fasciola gigantica* were selected from 55 cattle and were divided randomly into two groups (A, B), each group consisting of five cattle. Cattle of group A were treated with Oxyclozanide (Tremacid®,Renata Ltd., @ 15 mg/kg body weight) orally. Cattle of group B were kept as the infected control group without giving any treatment. Before trials (pre-treatment/day 0) with Oxyclozanide, initial body weight, total egg counts of liver fluke and hematological parameters were examined and recorded. During the experimental period, the faecal samples were examined on 7th, 14th, 21st and 28th day. Clinical parameters (body weight)

were also examined on day 0 and 28. The hematological parameters (TEC, Hb, ESR, TLC and PCV) were also examined on day 7th, 14th, 21st and 28th for the determination of effects of Oxyclozanide (Tremacid®).

Experimental Drug, Dose and Route

Group	Compositions	Preparation	Name of drug	Manufacturing company	Dose and route
A	Oxyclozanide	Bolus	Tremacid®	Renata Ltd. Bangladesh	@ 15 mg/kg body weight orally
B	Untreated infected control	-	-	-	-

Layout of Experiment

Group of cattle	Drug used	Days Pre treatment	Post-treatment			
		Day 0	Day 7	Day 14	Day 21	Day 28
A	Oxyclozanide (Tremacid®)	** ♦♦ ψψ	** ♦♦	** ♦♦	** ♦♦	** ♦♦ ψψ
B	Untreated infected control	** ♦♦ ψψ	** ♦♦	** ♦♦	** ♦♦	** ♦♦ ψψ

** Faecal sample examination
♦♦ Hematological tests (TEC, Hb, ESR, TLC and PCV)
ψψ Clinical parameter (body weight)

2.7. Faecal sample examination

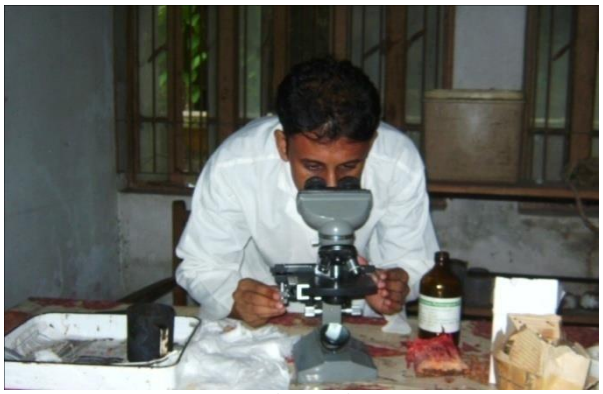
Faecal samples were collected directly from the rectum of each cattle. The samples were numbered according to the tag number of the cattle. In an extra sheet, tag number of the cattle, date of collection, age of the animals, health condition and other particulars were recorded. Immediately after collection samples were sent to the laboratory for examination by modified Stoll's Dilution Method (Soulsby, 1986).

2.7.1. Stoll's egg counting method (modified)

This is simple dilution procedure, which facilitates the recognition of eggs and larvae and permits a quantitative determination of their concentration in the faeces.

Procedure

a) 3 gm of faeces was mixed well and put in 100 ml beaker containing 42 ml of distilled water.
b) Some glass beads were added to it.
c) Then it was thoroughly mixed with stirrer.
d) The mixture was stained through a coffee stirrer.
e) The stained mixture was shaken, and 0.15 ml was taken with a small syringe. Put on a glass slide and covered with a 22×22 mm cover slip.
f) The slide was placed under microscope and whole of the 0.15 ml sample of suspension was examined under low power of the microscope (17 mm objective and x six oculars). The parasitic eggs were identified based on their characteristic morphological features.
g) The figures obtained from the count (i.e. the total number of eggs present in the 0.15 ml of diluted faeces) were multiplied by 100 to determine the number of eggs per gm (EPG) of the original faecal sample.



Pho. 1: Collection of Blood from Jugular Vein.



Pho. 2: Identification of Egg of Parasites under Microscope.

2.8. Determination of haematological parameters

Collection of blood

For the hematological examination, blood was collected aseptically with sterile syringe and needle from the jugular vein of cattle. Approximately 5 ml of blood was collected from jugular vein of each animal and was transferred immediately to a clean, dried glass vial containing anticoagulant (Sodium citrate) on day 0 (pretreatment) and 7th, 14th, 21st and 28th day of the post-treatment period. Then the collected blood samples were shifted to the laboratory in the CDIL, Dhaka. The hematological studies were performed within five hours after collection of blood. The routine analysis of blood was carried out by the standard method as described by Coffin (1955) and Schalm (1965). The following parameters were studied during the experimental period for fulfilling the objectives:

a) Total erythrocyte count (TEC)
b) Hemoglobin (Hb) content
c) Erythrocyte Sedimentation Rate (ESR)
d) Total leukocyte count (TLC)
e) Packed cell volume (PCV)

2.8.1. Total erythrocyte count (TEC)

Procedure

a) The tip of the dry cleans red blood cell pipette was placed on the blood.
b) The blood was sucked gently up until it reached the exactly 0.5 mark.
c) The tip of the pipette was wiped with a piece of cotton carefully.
d) Then the tip of the pipette was placed immediately in the diluting fluid (Hayem's Solution) and the pipette was filled exactly up to 101 marks.
e) The rubber tube was stretched around the tip of the pipette and held with thumb and finger at each end.

f) The content of the pipette was shaken thoroughly with eight knot motion for 1-2 minutes.
g) The counting chamber with cover glass was placed under the microscope, and visible rolled area was focused with a low power objective (X 10).
h) After discarding 2-3 drops, a small drop from the pipette was placed to the end of polished surface of the counting chamber and allowed the liquid to fill the area under cover glass.
i) The counting chamber was allowed to stand for a minute to allow the erythrocyte to settle.
j) Then the cells were started to count with the high power objective (45x).
k) The central primary square of the counting chamber were used for an erythrocyte count.
l) Red blood cells were counted in the four corner secondary squares and one center square of the secondary square of the chamber.

The number of RBC was calculated as follows
Number of RBC = No. of count × 10000 and the results expressed in million per cu. mm.

2.8.2. Hemoglobin (HB) content

Procedure

a) N/10 HCI solution was taken in the perfectly clean and dry special graduated tube up to its 2 gm % mark.
b) The special Sahli pipette was filled with blood up to 20 marks and wiped its side with absorbent cotton.
c) Immediately the blood of the pipette was transferred into the diluting tube containing N/10 HCI solution and the pipette rinsed 2-3 times by sucking water into the pipette, and this water added to the solution in the tube.
d) The tube was shaken until the blood was well mixed with N/10 HCI solution and water, and the mixture appeared uniformly dark-brown color.
e) Using the dropper, water was added drop by drop each time mixing the solution with a stirrer until color of the solution matched with the standard.
f) After 5 minutes of first noting time the result was read in a day light from the scale of diluting tube by observing the graduated mark at the lower edge of the meniscus at the top of the liquid column.
g) The result was expressed in gm %.

2.8.3. Erythrocyte sedimentation rate (ESR)

Procedure

a) The citrated blood was drawn into the special loading pipette.
b) The tip of the pipette was inserted to the bottom of a clean, dry Wintrobe heamatocrit tube.
c) The rubber bulb of the pipette was pressed continuously to expel the blood out of the pipette.
d) The Wintrobe heamatocrit tube was filled from the bottom.
e) As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of the blood to avoid foaming.
f) The tube was filled exactly up to 0 of the left sided scale.
g) The filled tube was kept standing in a vertical position on a standing rack for an hour.
h) After elapsing one hour the reading was taken from the scale at the top of the tube and the result was expressed in a millimeter in first hour.

2.8.4. Total leukocyte count (TLC)

Procedure

The principles of counting TLC were almost same to those of erythrocytes. Here the leukocyte diluting fluid was N/10 HCI solution. Well mixed blood was drawn up to the 0.5 mark of white blood cell pipette. The diluting fluid was filled up to the 11 mark of the pipette, and the content was thoroughly mixed for 2 minutes. 2-3 drops of content were discarded and counting chamber was then filled in the same way as in the red blood cell count. The counting chamber was placed under the microscope and examined under low power objectives (10x). The leukocytes in the four large squares (each one square mm.) of the counting, chamber were counted.
The number of W.B.C was calculated as follows:
Number of WBC = No. of cell counted × 50 and expressed the result in thousand per cu. mm.

2.8.5. Packed cell volume (PCV)

Procedure

a) The citrated blood was drawn into the special loading pipette.
b) The tip of the pipette was inserted to the bottom of a clean, dry Wintrobe heamatocrit tube.
c) The rubber bulb of the pipette was pressed continuously to expel the blood out of the pipette.
d) The Wintrobe heamatocrit tube was filled from the bottom. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of the blood to avoid air bubble.
e) The tip was filled exactly to the 10 mark of the right sided scale excess blood above the mark was wiped away by means of cotton.
f) The tubes were then placed in a centrifuge machine and centrifuged for 30 minutes at 3000 rpm.
g) After 30 minutes the tubes were taken out of centrifuge machine and PCV was read directly of the calibration on the right side of the tube.
h) The result was expressed in percentage (%) using the formula: PCV= weight of the packed Red cell (in cm)/ weight of the total blood in the tube (cm) X 100

2.9. Measurement of body weight

The body weight of all experimental cattle was taken on day '0" and 28th day of experiment. The body weight of each cattle was measured as per method cited by Samad (1996)

$$\text{Body weight} = \frac{\text{Length} \times (\text{Girth})^2}{300 \times 2.2} \text{ kg}$$

Here Length = Length from the point of shoulder to the buttock in inches.
Girth was also measured in inches at the point of xyphoid cartilage.

2.10. Analysis of the result and calculation

The data were analyzed statistically by using student "T" test (Gupta, 1978).
The percentage of reduction of EPG was calculated as

$$\frac{N_1 - N_2}{N_1} \times 100$$

N1= Number at day "0"
N2 = Number on next counting day

3. Results

The research work was conducted to evaluate the efficacy of Oxyclozanide (Tremacid®) against liver fluke infection (fascioliasis) for a period of 28 days in 10 cattle, out of 55. Each group consisted of 5 cattle. Cattle of group A were treated with Oxyclozanide bolus (Tremacid®, @ 15 mg/kg body weight). Cattle of group B was kept as infected control group without giving any treatment.

Attempts were also made to investigate the effects of the Oxyclozanide (Tremacid®) to determine some haematological parameters (TEC, Hb, ESR, TLC and PCV) and clinical parameter (body weight) of cattle.

3.1. Efficacy of oxyclozanide (Tremacid®) against liver fluke infection (fascioliasis) in cattle

The results of the efficacy of Oxyclozanide (Tremacid®) against liver fluke infection (fascioliasis) in cattle are shown in Table 1 Fig. 1. A significant ($p<0.01$) reduction of EPG count was found on 7th, 14th, 21st and 28th day of Tremacid® treated cattle of the group A. The EPG count of the untreated control group (group B) were significantly ($p<0.01$) increased seven-day onwards up to an experimental period.

In group A: Mean EPG count before treatment was 278.00±8.0 and after treatment with Tremacid® , the mean EPG on 7th, 14th, 21st and 28th day were 106.00±2.92, 80.00±3.54, 64.00±1.87 and 43.00±2.00 respectively. Reduction of mean EPG on 7th, 14th, 21st and 28th day after treatment were 61.87%, 71.22%, 76.98% and 84.53% respectively.

In group B: The Mean EPG of an untreated infected control group on pre-treatment (day 0) was 290.00±7.07. The mean EPG on 7th, 14th, 21st and 28th day were 302.00±8.00, 314.00±7.48, 326.00±7.48 and 3.38±5.83 respectively. Increased percent of mean EPG on 7th, 14th, 21st and 28th day-after treatments were 3.97%, 7.64%, 11.04% and 14.20% respectively.

3.2. Efficacy of oxyclozanide (Tremacid®) on haematological parameters in cattle

3.2.1. Total erythrocyte count (million/cu. mm.)

Tremacid® caused significant changes on total erythrocyte count (TEC). The TEC was increased significantly ($p<0.01$ and $P<0.05$) after Tremacid® treatment in the group A. Mean TEC before treatment was 7.48±0.08 and after treatment with Tremacid®, the mean TEC on 7th, 14th, 21st and 28th day were 7.64±0.07, 7.76±0.08, 7.88±0.07 and 7.94±0.07in group A respectively. Mean TEC of the untreated infected control group on pre-treatment (day 0) was 7.56±0.11. Mean TEC on the 7th, 14th, 21st and 28th day were 7.46±0.08, 7.32±0.10, 7.20±0.07 and 7.02±0.09 in group B respectively. The results of the effect of Oxyclozanide (Tremacid®) on Total erythrocyte count (TEC) are shown in the Table 2 and Fig. 2.

Table 1: Efficacy of Oxyclozanide (Tremacid®) against liver fluke infection (Fascioliasis) in Cattle

Groups	Drug with Dose	Pre-treatment EPG at '0' day Mean ±SE	Post treatment EPG at 7th day Mean ±SE	EPG at 14th day Mean ±SE	EPG at 21st day Mean ±SE	EPG at 28th day Mean ±SE
C	Oxyclozanide (Tremacid® -1000 mg/Tab) 15 mg/kg b.wt. orally	278.00 ± 8.00	106.00** ±2.92 (61.87%)	80.00** ± 3.54 (71.22%)	64.00** ± 1.87 (76.98%)	43.00** ± 2.00 (84.53%)
D	Control	290.00 ± 7.07	302.00** ±8.00 (3.97%)	314.00** ± 7.48 (7.64%)	326.00** ± 7.48 (11.04%)	338** ± 5.83 (14.20%)

Within the parenthesis value showing (% increase and decrease)
The above values represent the mean±SE of 5 cattle
** = Significant at 1 percent level ($p<0.01$)
* = Significant at 5 percent level ($p<0.05$)

Table 2: The Efficacy of Oxyclozanide (Tremacid®) at recommended doses on TEC (million/cu.mm.) in Cattle

Groups	Drug with dose	Pre-treatment TEC at 0 day Mean±SE	Post treatment TEC at 7th day Mean±SE	TEC at 14th day Mean±SE	TEC at 21st day Mean±SE	TEC at 28th day Mean±SE
A	Oxyclozanide (Tremacid® -1000 mg/Tab) 15 mg/kg b.wt orally	7.48 ± 0.08	7.64** ± 0.07 (2.09%)	7.76** ± 0.08 (3.61%)	7.88** ± 0.07 (5.08%)	7.94** ± 0.07 (5.79%)
B	Control	7.56 ± 0.11	7.46* ± 0.08 (1.32%)	7.32** ± 0.10 (3.17%)	7.20** ± 0.07 (4.76%)	7.02** ± 0.09 (7.14%)

Within the parenthesis value showing (% increase and decrease)
The above values represent the mean±SE of 5 cattle
** = Significant at 1 percent level ($p<0.01$)
* = Significant at 5 percent level ($p<0.05$)

3.2.2. Hemoglobin content (gm%)

The hemoglobin content (Hb) was also increased significantly ($p<0.01$) after Tremacid® treatment in the group A. Mean Hb content before treatment was 8.60±0.40 and after treatment with Tremacid® on 7th, 14th, 21st and 28th day were 8.90±0.29, 9.10±0.40, 9.60±0.40 and 9.70±0.20 in group A respectively. Mean Hb of the untreated infected control group on pretreatment (day 0) was 8.70±0.46. Mean Hb content on the 7th, 14th, 21st and 28th day were 8.40±0.37, 8.20±0.34, 8.10±0.43 and 7.90±0.37 in group B respectively. The results of the effect of Oxyclozanide (Tremacid®) on Hemoglobin content (Hb) are shown in the Table 3 and Fig. 3.

3.2.3. Erythrocyte sedimentation rate (mm in 1st hour)

The Erythrocyte sedimentation rate (ESR) was decreased significantly ($p<0.05$) after Tremacid® treatment in the group A. Mean ESR before treatment was 1.14±0.07 and after treatment with Tremacid®, the mean ESR on 7th, 14th, 21st and 28th day were 1.06±0.07, 0.98±0.06, 0.94±0.07 and 0.86±0.07in group A respectively. Mean ESR of the untreated infected control group on pre-treatment (day 0) was 1.08±0.07. Mean ESR on the 7th, 14th, 21st and 28th day were 1.12±0.06, 1.14±0.06, 1.14±0.06 and 1.20±0.04 in group B respectively. The results of the effect of Oxyclozanide (Tremacid®) on Erythrocyte sedimentation rate (ESR) are shown in the Table 4 and Fig. 4.

3.2.4. Total leukocyte count (thousand/cu.mm.)

The Total leukocyte count (TLC) was changed significantly ($p<0.01$) after Tremacid® treatment in the group A. Mean TLC before treatment was 8.18±0.04 and after treatment with Tremacid®, the mean TLC on 7th, 14th, 21st and 28th day were 7.88±0.04, 7.92±0.04, 7.94±0.02 and 7.98±0.04in group A respectively. Mean TLC of the untreated infected control group on per-treatment (day 0) was 7.98±0.08. Mean TLC on the 7th, 14th, 21st and 28th day were 8.08±0.07, 8.14±0.05, 8.22±0.04 and 8.26±0.04 in group B respectively. The results of the effect of Oxyclozanide (Tremacid®) on Total leukocyte count (TLC) are shown in the Table 5 and Fig. 5.

3.2.5. Packed cell volume (%)

The Packed cell volume (PCV) was increased significantly ($p<0.01$) after Tremacid® treatment in group A. Mean PCV before treatment was 29.30±0.62 and after treatment with Tremacid® , the mean PCV on 7th, 14th, 21st and 28th day were 29.90±0.58, 30.50±0.57, 30.90±0.64 and 31.40±0.53 in group C respectively. Mean PCV of untreated infected control group on pre-treatment (day 0) was 29.40±0.66. Mean PCV on the 7th, 14th, 21st and 28th day were 29.40±0.58, 29.00±0.72, 28.80±0.60 and 28.50±0.67 in group B respectively. The results of the effect of Oxyclozanide (Tremacid®) on packed cell volume (PCV) are shown in the Table 6 and Fig. 6.

3.3. Effects of oxyclozanide (Tremacid®) on body weight (kg) in cattle

The body weight was increased significantly ($p<0.01$ and $p<0.05$) after Oxyclozanide (Tremacid®) treatment in the group A. Showed 28 days post treatment effect of Tremacid® on body weight of cattle. There was significant improvement in body weight following the administration of drugs. However, the highest improvement was observed on 28th day of post-treatment of drugs, and the percentage of improvement was 1.95% in the group of A respectively. Whereas body weight was reduced to the extent of 1.08% in the control group B after 28 days. The results of the effect of Oxyclozanide (Tremacid®) on body weight are shown in the Table 7 and Fig. 7.

Table 3: The Efficacy of Oxyclozanide (Tremacid®) at recommended doses on Hb content (gm %) in Cattle

Groups	Drug with dose	Pre-treatment Hb at 0 day Mean ±SE	Post treatment Hb at 7th day Mean ±SE	Hb at 14th day Mean ±SE	Hb at 21st day Mean ±SE	Hb at 28th day Mean ±SE
A	Oxyclozanide (Tremacid® -1000 mg/Tab) 15 mg/kg b.wt orally	8.60 ± 0.40	8.90 ± 0.29 (3.37%)	9.10* ± 0.40 (5.49%)	9.60* ± 0.40 (14.58%)	9.70* ± 0.20 (17.17%)
B	Control	8.70 ± 0.46	8.40 ± 0.37 (3.45%)	8.20* ± 0.34 (5.75%)	8.10* ± 0.43 (6.90%)	7.90* ± 0.37 (9.20%)

Within the parenthesis value showing (% increase and decrease)
The above values represent the mean±SE of 5 cattle
** = Significant at 1 percent level ($p<0.01$), * = Significant at 5 percent level ($p<0.05$)

Table 4: The Efficacy of Oxyclozanide (Tremacid®) at recommended doses on ESR (mm/1st hour) in Cattle

Groups	Drug with dose	Pre-treatment ESR at 0 day Mean ±SE	Post treatment ESR at 7th day Mean ±SE	ESR at 14th day Mean ±SE	ESR at 21st day Mean ±SE	ESR at 28th day Mean ±SE
A	Oxyclozanide (Tremacid® -1000 mg/Tab) 15 mg/kg b.wt orally	1.14 ± 0.07	1.06* ± 0.07 (7.02%)	0.98** ± 0.06 (14.04%)	0.94** ± 0.07 (17.54%)	0.86** ± 0.07 (24.56%)
B	Control	1.08 ± 0.07	1.12 ± 0.06 (3.57%)	1.14 ± 0.06 (5.26%)	1.14 ± 0.06 (5.26%)	1.20 ± 0.04 (10.00%)

Within the parenthesis value showing (% increase and decrease)
The above values represent the mean±SE of 5 cattle
** = Significant at 1 percent level ($p<0.01$), * = Significant at 5 percent level ($p<0.05$)

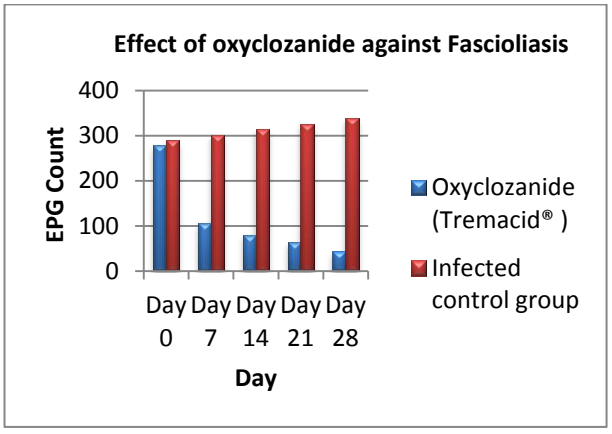


Fig. 1: The Efficacy of Oxyclozanide (Tremacid®) against liver fluke infection (Fascioliasis) in Cattle.

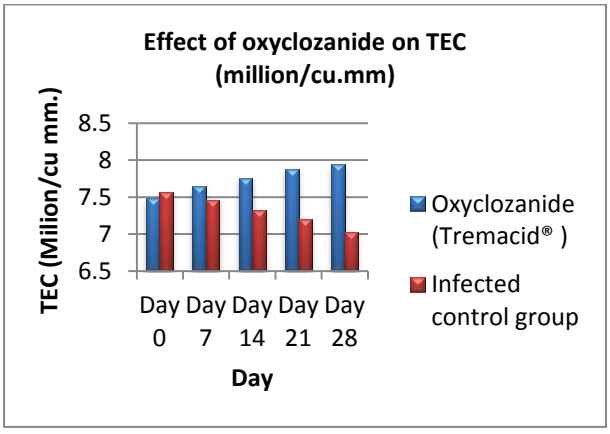


Fig. 2: The Efficacy of Oxyclozanide (Tremacid®) at recommended doses on TEC (million/cu.mm.) in Cattle.

Table 5: The Efficacy of Oxyclozanide (Tremacid®) at recommended doses on TLC (thousand/cu.mm.) in Cattle

Groups	Drug with dose	Pre-treatment TLC at 0 day Mean ±SE	Post treatment TLC at 7th day Mean ±SE	TLC at 14th day Mean ±SE	TLC at 21st day Mean ±SE	TLC at 28th day Mean ±SE
A	Oxyclozanide (Tremacid® -1000 mg/Tab) 15 mg/kg b.wt orally	8.18 ± 0.04	7.88** ± 0.04 (3.67%)	7.92** ± 0.04 (3.18%)	7.94* ± 0.02 (2.93%)	7.98 ± 0.04 (2.44%)
B	Control	7.98 ± 0.08	8.08* ± 0.07 (1.24%)	8.14* ± 0.05 (1.97%)	8.22** ± 0.04 (2.92%)	8.26** ± 0.04 (3.39%)

Within the parenthesis value showing (% increase and decrease)
The above values represent the mean±SE of 5 cattle
** = Significant at 1 percent level ($p<0.01$), * = Significant at 5 percent level ($p<0.05$)

Table 6: The Efficacy of Oxyclozanide (Tremacid®) at recommended doses on PCV (%) in Cattle

Groups	Drug with dose	Pre-treatment PCV at 0 day Mean ±SE	Post treatment PCV at 7th day Mean ±SE	PCV at 14th day Mean ±SE	PCV at 21st day Mean ±SE	PCV at 28th day Mean ±SE
A	Oxyclozanide (Tremacid® - 1000 mg/Tab) 15 mg/kg b.wt. orally	29.30 ± 0.62	29.90* ± 0.58 (2.01%)	30.50** ± 0.57 (3.93%)	30.90** ± 0.64 (5.18%)	31.40* ± 0.53 (6.37%)
B	Control	29.40 ± 0.66	29.40 ± 0.58	29.00* ± 0.72 (1.36%)	28.80* ± 0.60 (2.04%)	28.50* ± 0.67 (3.06%)

Within the parenthesis value showing (% increase and decrease)
The above values represent the mean±SE of 5 cattle
** = Significant at 1 percent level (p<0.01)
* = Significant at 5 percent level (p<0.05)

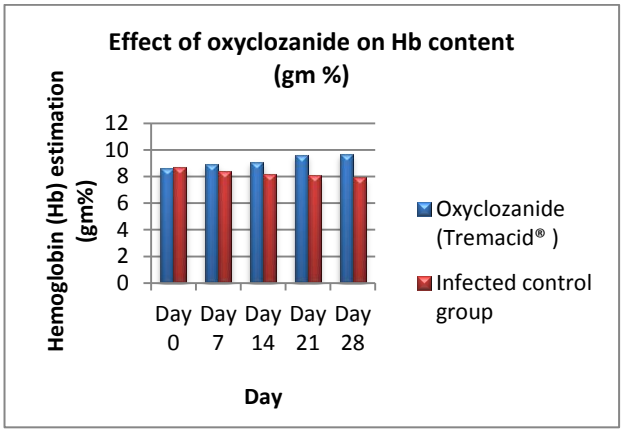


Fig. 3: Efficacy of Oxyclozanide (Tremacid®) ® at recommended doses on Hb content (gm %) in Cattle.

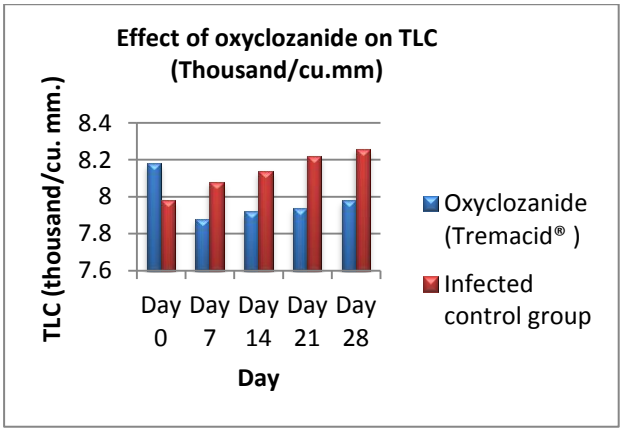


Fig. 5: Efficacy of Oxyclozanide (Tremacid®) at recommended doses on TLC (thousand/cu.mm) in Cattle.

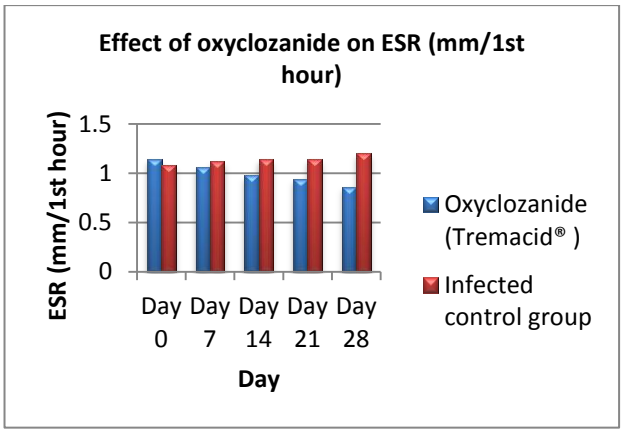


Fig. 4: The Efficacy of Oxyclozanide (Tremacid®) at recommended doses on ESR (mm/1st hour) in Cattle

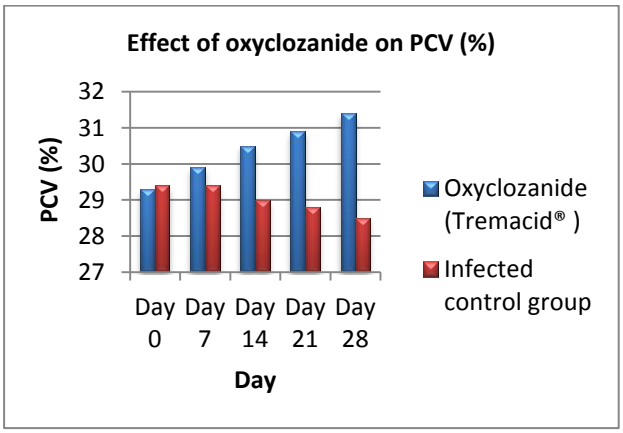


Fig. 6: Efficacy of Oxyclozanide (Tremacid®) at recommended doses on PCV (%) in Cattle

Table 7: The Efficacy of Oxyclozanide (Tremacid®) at recommended doses on Body Weight (kg) gain/loss in Cattle

Groups	Drug with dose	Pre-treatment 0 day (body weight) Mean ±SE	Post treatment 28th day (body weight) Mean ±SE	Live weight gain/loss (kg)	Improvement (%)
A	Oxyclozanide (Tremacid® - 1000 mg/Tab) 15 mg/kg body weight orally	143.80±3.58	146.60** ± 3.71 (1.95%)	+2.80	+1.95%
B	Control	148.20±4.18	146.60** ± 4.25 (1.08%)	-1.60	-1.08%

Within the parenthesis value showing (% increase and decrease)
The above values represent the mean±SE of 5 cattle
** = Significant at 1 percent level (p<0.01)
* = Significant at 5 percent level (p<0.05)

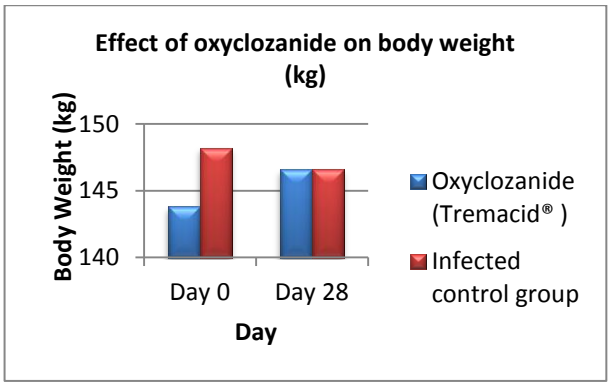


Fig. 7: Efficacy of Oxyclozanide (Tremacid®) at recommended doses on Body Weight (kg) in Cattle.

4. Discussion

Fascioliasis has been implicated as the cause of morbidity and mortality in the production of ruminants (Okoli 2001). Fascioliasis is a trematode borne parasitic disease that infects the liver of large ruminants widely prevalent throughout the world. It is caused by Fasciola spp. i.e., *Fasciola gigantica*, and *Fasciola hepatica* (Phiri et al 2006). The experiment was conducted for a period of 28 days to study the effects of Tremacid® against liver fluke infection (fascioliasis) in 10 cattle. Fascioliasis is one of the major parasitic diseases of ruminants affecting livers and gall bladders of cattle. Parasitic diseases not only cause mortality of animals, and also have direct effects in term of reduced production of milk, meat, wool, hide production; condemnation of liver, loss of draught power, reproductive failure and mortality (Rahman and Rahman 1972, Fabiyi 1986, Diaw et al 1998), infertility and loss of stamina of working animals and especially zoonotic impact on human health are considerably greater (Baker & Muller, 1988). Helminthiasis, pose a serious health threat and a limitation to the productivity of ruminants due to the associated morbidity, mortality, cost of treatment and control measures (Nwosu et al 2007).

In Bangladesh, it is understood that cattle is reared without proper management. Moreover, the geographical and topographical condition of Bangladesh favors the growth and multiplication of helminths in cattle. So percentage of mixed parasitic infection in cattle of Bangladesh is high. The efficacy of Tremacid® was evaluated on the basis of the percentage to reduction in mean egg count compared to the mean egg count per gram of faeces. Considering the limitations in interpreting results and on the basis of faecal egg count, it could be said that it would be ideal to do a post-mortem parasite count after treatment. And for a detailed and crucial experimental study, it was absolutely necessary to do actual parasite count rate than faecal egg count. Nevertheless, the number of eggs in the faeces was a reliable indication of the actual number of parasite in the host. Not only the EPG count, the effects on TEC, Hb, PCV, ESR, TLC and body weight due to Oxyclozanide (Tremacid®) treatment also compared to the pre-treatment value in cattle.

4.1. Efficacy of oxyclozanide (Tremacid®) against liver fluke infection (fascioliasis) in cattle

A significant ($p<0.01$) reduction of EPG count was found on 7th, 14th, 21st and 28th day of Tremacid® treated cattle of the group C. The EPG count of the control group were significantly ($p<0.01$) increased seven-day onwards up to an experimental period.

Mean EPG count before treatment was 278.00±8.00 and after treatment with Tremacid® mean EPG on 7th, 14th, 21st and 28th day were 106.00±2.92, 80.00±3.54, 64.00±1.87 and 43.00±2.00 respectively. Reduction of mean EPG on 7th, 14th, 21st and 28th day after treatment were 61.87%, 71.22%, 76.98% and 84.53% respectively. The similar findings were also reported by Babicek et al (1993), Coles and Stafford (2001) in sheep. The findings was also in agreement with the works of Richards et al. (1990), Waruiru et al. (1994), Sahoo et al (2002), Gupta and Singh (2002). Likewise, Ratnaparkhi et al (1992), Ratnapakhi et al (1993), Prased et al (2001), Gupta and Singh (2002) observed similar results in buffaloes.

The Mean EPG of the untreated infected control group (B) on pre-treatment (day 0) was 290.00±7.07. The mean EPG on 7th, 14th, 21st and 28th day were 302.00±8.00, 314.00±7.48, 326.00±7.48 and 338±5.83 respectively. Increased percentage of mean EPG on 7th, 14th, 21st and 28th day-after treatment were 3.97%, 7.64%, 11.04% and 14.20% respectively. The present finding was in agreement with the work of Gupta (1988) in buffaloes, Coles and Stafford (2001) in lamb. Paraud et al (2009) also reported that the efficacy of oxyclozanide was 96% in goat and concluded that oxyclozanide is highly effective in reducing the number of rumen flukes. This study supports the previous findings recorded by Islam and Samad (1989). Rapic et al (1988), Richards et al (1990) reported that the anthelmintics showed the better efficacy against liver fluke in cattle. Mooney et al (2009) also reported more than 98% efficacy in a hill sheep flock in the west of Ireland. Queiroz et al 2013 reported on antihelmintic that is utilized in ruminants for the control of trematodes, particularly for Fasciola hepatic.

4.2. Efficacy of oxyclozanide (Tremacid®) on haematological parameters in cattle

4.2.1. Total erythrocyte count (million/cu.mm.)

Tremacid® caused significant changes on total erythrocyte count (TEC).The The TEC was increased significantly ($p<0.01$) after Tremacid® treatment in the group A. Mean TEC before treatment was 7.48±0.08 and after treatment with Tremacid® mean TEC on 7th, 14th, 21st and 28th day were 7.64±0.07, 7.76±0.08, 7.88±0.07 and 7.94±0.07in group A respectively. Mean TEC of the untreated infected control group on per-treatment (day 0) was 7.56±0.11. The findings were also in agreement with the works of Mason and Offer (2003), Kamruzzaman (2004) in cattle. Mean TEC on the 7th, 14th, 21st and 28th day were 7.46±0.08, 7.32±0.10, 7.20±0.07 and 7.02±0.09 in group B respectively. The improved level of TEC content of blood in treated cattle might be due to elimination of liver fluke (*Fasciola gigantica*).

4.2.2. Haemoglobin content (gm %)

The haemoglobin content (Hb) was also increased significantly ($p<0.01$) after Tremacid® treatment in group A respectively. Mean Hb content before treatment was 8.60±0.40 and after treatment with Tremacid®, the mean Hb on 7th, 14th, 21st and 28th day were 8.90±0.29, 9.10±0.40, 9.60±0.40 and 9.70±0.20 in group A respectively. The similar findings were also reported by Widjajanti et al (2001), Kamruzzaman (2004) in cattle. Mean Hb of the untreated infected control group on per-treatment (day 0) was 8.70±0.46. Mean Hb content on the 7th, 14th, 21st and 28th day were 8.40±0.37, 8.20±0.34, 8.10±0.43 and 7.90±0.37 in group B respectively. The increase in hemoglobin content may be due to the increase of total erythrocyte count (TEC).

4.2.3. Erythrocyte sedimentation rate (mm in 1st hour)

The Erythrocyte sedimentation rate (ESR) was decreased significantly ($p<0.05$) after Tremacid® treatment in group A. Mean ESR before treatment was 1.14±0.07 and after treatment with Tremacid® mean ESR on 7th, 14th, 21st and 28th day were 1.06±0.07, 0.98±0.06, 0.94±0.07 and 0.86±0.07 in group A respectively. The finding was also in agreement with the works of Widjajanti et al (2001), Mason and Offer (2003), Kamruzzaman (2004) in cattle. Mean ESR of untreated infected control group on per-treatment (day 0) was 1.08±0.07. Mean ESR on the 7th, 14th, 21st and 28th day were 1.12±0.06, 1.14±0.06, 1.14±0.06 and 1.20±0.04 in group B respectively.

4.2.4. Total leukocyte count (thousand/cu.mm.)

The Total leukocyte count (TLC) was increased significantly ($p<0.01$) after Tremacid® treatment in the group A. Mean TLC before treatment was 8.18±0.04 and after treatment with Tremacid®, the mean TLC on 7th, 14th, 21st and 28th day were 7.88±0.04, 7.92±0.04, 7.94±0.02 and 7.98±0.04 in group A respectively. Mean TLC of the untreated infected control group on pertreatment (day 0) was 7.98±0.08. Mean TLC on the 7th, 14th, 21st and 28th day were 8.08±0.07, 8.14±0.05, 8.22±0.04 and 8.26±0.04 in group B respectively. Similar activity of oxyclozanide in buffalo, cattle, sheep and goats naturally infected with *Fasciola gigantica* by Roy and Sukhla (1971).

4.2.5. Packed cell volume (%)

The Packed cell volume (PCV) was increased significantly ($p<0.01$) after Tremacid® treatment in the group A. Mean PCV before treatment was 29.30±0.62 and after treatment with Tremacid® mean PCV on 7th, 14th, 21st and 28th day were 29.90±0.58, 30.50±0.57, 30.90±0.64 and 31.40±0.53 in group A respectively. Mean PCV of the untreated infected control group on pertreatment (day 0) was 29.40±0.66. Mean PCV on the 7th, 14th, 21st and 28th day were 29.70±0.58, 29.00±0.72, 28.80±0.60 and 28.50±0.67 in group B respectively. Similarly oxyclozanide drug used by Hiepe et al (1970) for the bovine facioliasis.

4.3. Effects of oxyclozanide (Tremacid®) on body weight (kg) in cattle

The body weight was increased significantly ($p<0.01$) after Tremacid® treatment in the group A. Showed 28 days post treatment effect of Tremacid® on body weight of cattle). There was a significant improvement in body weight following the administration of drugs. However, the highest improvement was observed on 28th day of post-treatment of drugs, and the percentage of improvement was 1.95% in the group of A. Whereas, body weight was reduced to the extent of 1.08% in the control group B after 28 days.

This result was support by Sanchez et al (1988), Asaduzzaman (1998), Isles et al (1985), Mason and Offer (2004), Kamruzzaman (2004), Mcconville et al (2006), Richards et al (2009). Due to removal of parasitic load might have had facilitated the regain through proper digestion, absorption and metabolism of feed nutrient in the liver fluked (*Fasciola gigantica)* free cattle. Fascioliasis is cosmopolitan infection. Incidence o f the infection has been reported in many countries, including Nigeria, Parkistan, China, United States of America and Iran staed by Valero et al (2010) and WHO (2006). It is commonly reported in ruminants; cattle, goat and sheep (Okaiyeto et al 2012, Talukder et al 2010, Ozung et al 2011). Soulsby (1986) reported that this group of liver fluke (Fasciola sp.) is also associated with anaemia resulting loss of body weight, stunted growth, diarrhea etc. that greatly hamper the normal growth and production of cattle. The body weight was increased, and this may be due to removal of parasitic load, proper absorption and metabolism of nutrient in the parasite free gastrointestinal tract. The body weight gains are supported by Isles et al (1985) in heifers. On the other hand, the body weight significantly decreased in the untreated control group due to overload of parasites within the body of cow (Chowdhury et al 2014).

5. Conclusion

The finding of the present study reveals that commercial product Oxyclozanide (Tremacid®) is effective for reduction of EPG of Fasioliasis. This drug has wide therapeutic index, and they may kill or inhibit egg production of liver fluke in cattle. However, the preliminary control efficacy studies of anthelmintic may help to explore the details of pharmacokinetic study. The findings of the present study reveal that Oxyclozanide (Tremacid®) is effective for reduction of EPG of liver fluke (Fasciola spp.). However, the present results are also preliminary control efficacy studies of parasitic infestation and anthelmintics, which may help the future researches to explore the detailed pharmacokinetic and toxic effects for wide therapeutic uses in Bangladesh for the treatment of parasitic infection in other animals.

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