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Evaluation of oxyclozanide and niclosamide combination as alternative antiparamphistomal therapy in buffaloes

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Paramphistomiasis causes enteritis and anemia in livestock and result in substantial production and economic losses. It is considered a neglected tropical disease, with lower effective trematodicidal compound for treatment. Keeping in view the importance of disease, the present study aims to evaluate the efficacy of oxyclozanide and niclosamide, as well as a combination of both drugs in treatment of *Paramphistomum* infection. Twenty buffalo males of native breed between eight months to 2 years of age, naturally infected with *Paramphistomes* were treated. Oxyclozanide and niclosamide were administered in 20 ml/100 kg and 125 mg/kg orally, respectively. The efficacies of the drugs were estimated on the bases of reduction in body weight, drug efficacy, paramphistomes egg count per gram feces, as well as, hematological and biochemical parameters. The obtained results revealed that Co administration of oxyclozanide and niclosamide resulted in amelioration of the most adverse effects associated with *Paramphistomum* infection and reflected significantly on decreased egg per gram count and oxidative stress, improved biochemical and hematological profiles.

Key words: oxyclozanide, niclosamide, antiparamphistomal, therapy, buffalo.

INTRODUCTION

Paramphistomiasis is largely a disease of young animals less than two years of age, because repeated infections of low intensity generally produce an almost complete immunity. Adult *Paramphistomes* are the main parasites in the rumen and reticulum of sheep, goats, cattle, and water buffaloes; the pathological effects of infection are almost entirely caused by the immature stages within the first part of the small intestine (Zahir et al., 2012). The immature worms penetrate the mucosa of the small intestine as deeply as the musculosa. This causes strangulation and the eventual necrosis of the piece of mucosa, leading to the development of erosions and petechiae. These lesions cause acute parasitic gastroenteritis with high morbidity and mortality rates, particularly in young animals (Rolfe and Boray, 1993). At the same time, hypoalbuminemia which is by losing seepage and other plasma protein into the gut, coupled with loss of appetite, seems to be the most important pathophysiological consequence of paramphistomiasis (Sissay, 2007). *Paramphistomum* infection provokes a lower feed conversion, a loss of weight, and/or a decrease in milk production, which results in economic loss (Rangel-Ruiz et al., 2003). *Paramphistomum cervi* is considered as one of the most important species of *Paramphistomes*, since they are cattle parasites with a cosmopolitan distribution (Hassan et al., 2005). Paramphistomiasis can be controlled by periodic treatment with a repertoire of drugs. Several drugs have been assessed and recommended

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for the treatment of paramphistomiasis (Panyarachun et al., 2010). Although treatment for adult fluke has no direct benefit to the animal, it may reduce the source of infection for the snail intermediate host. This then reduces the size of the next generation of infective fluke larvae on pasture (Soulsby, 1982). The current research mainly focused on developing alternative drug formulations and evaluates the effect of both oxyclozanide and niclosamide on *Paramphistomes* applied on live animals, especially, the buffaloes.

MATERIALS AND METHODS

Animals

Twenty buffalo males of native breed between eight months to 2 years of age naturally infected with *Paramphistomes*, based on their fecal examination by sedimentation and floatation techniques (Kruse and Pritchard, 1982). Use of animals in this study was in accordance with Good Laboratory Practice standards and national welfare regulations.

Drugs and treatment

Oxyclozanide (Zanil® Fluke Drench 3.40 w/v%) was obtained from Schering-Plough Egypt. The recommended dose is 20 ml/100 kg orally, with two treatments given two days apart (Rolfe and Boray, 1987). Niclosamide (Niclosan® 500) was obtained from Misr Co. For Pharm. Ind. Cairo, Egypt. The recommended dose is 125 mg/kg orally (Einstein et al., 1994) and repeated after 14 days (Bishop, 2005).
Animals were divided into 4 groups, each of 5 animals as follows:

Group 1. Buffaloes were treated with oxyclozanide at oral dose of 20 ml/100 kg. Two doses were given two days apart.
Group 2. Buffaloes were orally treated with niclosamide at a dose of 125 mg/kg and repeated after each 14 days (five times) to overcome the prepatent period of *Paramphistomes*, 7-10weeks (Gerold and Hannah, 2007).
Group 3. Buffaloes were orally treated with oxyclozanide at a dose of 20 ml/100 kg. Two doses were administered two days apart and after the last dose of oxyclozanide, niclosamide at a dose of 125 mg/kg was orally administered and repeated after each 14 days (five times).
Group 4. Buffaloes were left as control infected non treated animals monitored weekly.

The animals of all groups were weighted every two weeks during the trial period.

Fecal sampling and parasitological examination

One week before and at the time of treatment (zero day) later weekly throughout the treatment trials, fecal samples were collected from each animal. The examinations of the fecal samples were done by direct smear subsequently floatation technique and fecal culture (Kruse and Pritchard, 1982) to ensure the status of infection with other parasites. Sedimentation technique and eggs count (eggs per gram of feces, EPG) were determined by the McMaster technique (Rieu et al., 2007) for a fecal count of *Paramphistomes* eggs. A complete description of each case was recorded. The efficacies of the drugs used were evaluated according to the following equation recorded by Khayatnouri et al. (2011).

$$\% \text{ of drug efficacy} = P\text{-}R/P \times 100$$

Where, R, Average number of parasite egg in a gram of fecal sample after treatment; P = average number of parasite egg in a gram of fecal sample before treatment.

Blood sampling

Every two weeks during the experimental period (ten weeks), two venous blood samples (six ml) were taken from each animal. The first blood sample was collected in test tubes containing heparin for hematological studies. While the second blood samples were allowed to coagulate at 4 °C and were then centrifuged at 3000 rpm for 15 min to separate the serum. The serum samples were frozen at −20°C.

Hematological analysis

The hematological parameters, red blood cells (RBCs) count, hemoglobin (Hb%), and packed cell volume (PCV%) were estimated by using an automatic cell counter (Exigo, Veterinary Hematology System, Boule Medical AB, Stockholm, Sweden.).

Biochemical analysis

Total protein, blood albumin, glutathione, malondialdehyde, serum glucose, blood sodium and blood potassium were measured in serum by commercially available kit methods. Globulins were estimated by electrophoretic analysis of serum protein.

Statistical analysis

The descriptive data are presented as the means ± SE. The statistical differences were calculated on the basis of two way test of ANOVA and $p < 0.05$ is considered as significant between the groups. The data were statistically analyzed by using one way ANOVA test for variance analysis (Student-Newman-Keuls) at $p < 0.05$, using the SPSS 13.0 Windows statistical package (2004).

RESULTS

All animals were clinically healthy throughout the experiment. None of the buffaloes in all groups suffered from identifiable reactions following the administration of oxyclozanide or niclosamide or the combination of both drugs. Oxyclozanide at a dose of 20 ml/100 kg, where two doses were orally given two days apart and after the last dose of oxyclozanide, niclosamide at a dose of 125 mg/kg was orally given and repeated every 14 days (five times) revealed significant effects on body weight, number of egg count/gram and drug efficacy (%) (Table 1, 2 and 3).

Body weight

Beginning with the 4th week of the experiment the drug combination improved significantly ($p < 0.05$) the body weight (kg) of animals (331.14 ± 9.32) with respect to (312.27 ± 6.30: 309.15 ± 8.33 and 319.16 ± 4.22) in

Table 1. Effect of orally administered oxyclozanide (20 ml/100 kg) or niclosamide (125 mg/kg) and both drugs on body weight (kg) of buffaloes naturally infected with *Paramphistomes* (n = 5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	288.22±4.60^{g}	297.55±6.70^{g}	309.15±8.33^{f}	319.25±6.28^{f}	327.12±5.16^{ef}	339.10±8.19^{e}
2nd group	289.14±7.18^{g}	300.26±5.12^{fg}	312.27±6.30^{f}	323.45±5.11^{ef}	337.34±5.56^{e}	357.64±9.57^{d}
3rd group	291.14±3.16^{g}	312.18±4.22^{f}	331.14±9.32^{e}	356.26±6.23^{d}	371.27±6.53^{c}	394.27±6.53^{a}
Control	290.50±5.32^{g}	302.62±7.21^{fg}	319.16±4.22^{f}	324.00±4.12^{ef}	336.14±6.33^{e}	356.14±9.43^{d}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different ($p < 0.05$).

Table 2. *Paramphistomes* egg count before and during treatment with oxyclozanide (20 ml/100 kg), niclosamide (125 mg/kg) or both drugs.

Weeks / Groups	One week before	0 day	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week
1st group	5.30±0.13^{ab}	5.30±0.13^{ab}	2.50±0.11^{e}	0.60±0.004^{g}	1.00±0.004^{f}	1.30±0.01^{f}	1.16±0.002^{f}	1.30±0.10^{f}	1.30±0.10^{f}	2.10±0.11^{e}	1.60±0.11^{f}	2.80±0.10^{e}
2nd group	5.50±0.13^{ab}	5.47±0.12^{ab}	5.50±0.13^{ab}	4.66±0.02^{c}	4.63±0.01^{c}	5.10±0.27^{b}	4.80±0.17^{c}	4.30±0.11^{c}	4.30±0.01^{c}	5.00±0.16^{b}	4.30±0.21^{c}	3.80±0.16^{d}
3rd group	5.22±0.07^{b}	5.62±0.11^{a}	2.50±0.001^{e}	1.36±0.001^{f}	0.33±0.001^{g}	0.33±0.001^{g}	0.33±0.001^{g}	0.00±0.00^{h}	0.00±0.00^{h}	0.00±0.00^{h}	0.00±0.00^{h}	0.00±0.00^{h}
Control	5.31±0.20^{ab}	5.52±0.10^{ab}	5.50±0.11^{ab}	5.67±0.22^{a}	5.31±0.20^{ab}	5.22±0.07^{b}	5.16±0.14^{b}	5.45±0.15^{ab}	5.54±0.22^{ab}	5.00±0.11^{b}	5.44±0.10^{ab}	5.83±0.14^{a}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

control animals, respectively. At the end of the experiment, 10th week, the significant differences increased (394.27 ± 6.53) vs. (357.64 ± 9.57; 339.10 ± 8.19 and 356.14 ± 9.43) in control animals, respectively.

The number of *Paramphistomes* eggs /gram

During the preparation of the experiment, some nematode eggs were recorded in some cases with our target fluke (*Paramphistomum* spp.) eggs. And so, these animals were excluded from the experimental trial. Infection of naturally infected buffaloes with *Paramphistomes* was significantly decreased after treatment until the 6th week of the experiment (0.00 ± 0.00) then remains stable until the end of the experiment. On the other side, in the control positive non treated group, the number of eggs per gram remain noticed until the end of the experiment (3.80 ± 0.16; 2.80 ± 0.10 and 5.83 ± 0.14) in control animals, respectively. The effect of oxyclozanide appeared in the 2nd week (0.60±0.004), but the recurrent appearance of the eggs showed an ascending manner from the 2nd week of treatment until the end of the trial (2.80±0.10). In niclosamide treated group, the results showed no obvious reduction in eggs per gram. While the group treated with the drug combination showed an effective decrease in *Paramphistomes* eggs from the first week (2.50±0.001), and shedding of eggs was completely stopped at the 6th week (00 EPG) until the end of experimental trial.

Drug efficacy (%)

Drug efficacy (%) significantly improved through the fall of the *Paramphistomes* eggs count for the 6th week (100) and stabilized until the conclusion of the experiment (100) vs. another drug tested groups (0 and 59.25, respectively).

Hematological findings

Oxyclozanide at a dose of 20 ml/100 kg, where

Table 3. Effect of orally administered of oxyclozanide (20 ml/100kg) or niclosamide (125 mg/kg) and both drugs on drug efficacy (%)of buffaloes naturally infected with *Paramphistomes* (n=5).

Weeks / Groups	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week
1st group	62.9	77.77	77.77	70.3	70.3	66.66	66.66	59.25	66.66	59.25
2nd group	0	0	0	0	0	0	0	0	0	0
3rd group	80.76	96.15	96.15	96.15	96.15	100	100	100	100	100
Control	0	0	0	0	0	0	0	0	0	0

Table 4. Effect of orally administered of oxyclozanide (20 ml/100kg) or niclosamide (125 mg/kg) and both drugs on red blood cell count (x 10^6/ µl) of buffaloes naturally infected with *Paramphistomes* (n=5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	5.20 ± 0.15^{c}	5.40 ± 0.12^{bc}	5.42 ± 0.16^{bc}	5.44 ± 0.12^{bc}	5.44 ± 0.08^{bc}	5.40 ± 0.12^{bc}
2nd group	5.10 ± 0.13^{c}	5.50 ± 0.14^{bc}	5.50 ± 0.10^{bc}	5.70 ± 0.11^{b}	5.74 ± 0.10^{b}	5.78 ± 0.11^{b}
3rd group	5.10 ± 0.10^{c}	5.70 ± 0.13^{b}	5.70 ± 0.11^{b}	6.10 ± 0.12^{a}	6.08 ± 0.14^{a}	6.12 ± 0.10^{a}
Control	5.50 ± 0.22^{c}	5.56 ± 0.13^{bc}	5.50 ± 0.20^{bc}	5.60 ± 0.16^{b}	5.54 ± 0.11^{b}	5.60 ± 0.13^{b}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

Table 5. Effect of orally administered of oxyclozanide (20 ml/100kg) or niclosamide (125 mg/kg) and both drugs on hemoglobin values (g/dl) of buffaloes naturally infected with *Paramphistomes* (n = 5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	8.40 ± 0.11^{cd}	8.45 ± 0.18^{cd}	8.34 ± 0.14^{d}	8.36 ± 0.14^{d}	8.30 ± 0.14^{d}	8.21 ± 0.13^{d}
2nd group	8.55 ± 0.13^{c}	8.47 ± 0.15^{c}	8.44 ± 0.14^{c}	8.49 ± 0.14^{c}	8.49 ± 0.12^{c}	8.48 ± 0.17^{c}
3rd group	8.46 ± 0.16^{c}	8.72 ± 0.10^{b}	8.9 ± 0.14^{b}	9.0 ± 0.12^{a}	9.0 ± 0.12^{a}	8.9 ± 0.11^{ab}
Control	8.21 ± 0.19^{c}	8.14 ± 0.12^{c}	8.13 ± 0.12^{c}	8.27 ± 0.14^{c}	8.26 ± 0.11^{c}	8.29 ± 0.13^{c}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

Table 6. Effect of orally administered of oxyclozanide (20 ml/100kg) or niclosamide (125 mg/kg) and both drugs on packed cell volume values (%) of buffaloes naturally infected with *Paramphistomes* (n=5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	29.43 ± 0.25^{bc}	28.43 ± 0.88^{c}	28.76 ± 0.45^{c}	27.46 ± 0.66^{c}	28.75 ± 1.52^{c}	28.58 ± 0.42^{c}
2nd group	27.12 ± 0.55^{c}	27.27 ± 0.24^{c}	28.43 ± 0.74^{c}	28.08 ± 0.26^{c}	27.42 ± 1.43^{c}	29.92 ± 0.33^{c}
3rd group	28.36 ± 0.33^{c}	30.48 ± 0.33^{bc}	30.37 ± 0.36^{bc}	32.59 ± 0.74^{ab}	32.25 ± 1.73^{ab}	31.12 ± 0.46^{ab}
Control	26.44 ± 1.45^{c}	25.25 ± 2.14^{c}	26.42 ± 1.56^{c}	25.32 ± 1.75^{c}	25.62 ± 1.02^{c}	25.56 ± 1.43^{c}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

two doses were orally given two days apart and after the last dose of oxyclozanide, niclosamide at a dose of 125 mg/kg was orally given and repeated every 14 days (five times) revealed significant effects on red blood cell count, hemoglobin values and packed cell volume (%) (Table 4, 5 and 6). Beginning with the 6^{th} week of the experiment the drug combination improved significantly ($p < 0.05$) the red blood cell count (x10^6/µl) of animals (6.10 ± 0.12) and stabilized until the end of the experiment, 10^{th} week, 6.12 ± 0.10. Hemoglobin values (g/dl) improved significantly (p

< 0.05) by the 6th week of the experiment (9.0 ± 0.12) and continued until the end of the 10th week (8.9 ± 0.11) vs. treated groups. Packed cell volume values (%) improved significantly (p < 0.05) by the 6th week of the experiment (32.59 ± 0.74) and continued until the end of the 10th week (31.12 ± 0.46) vs. treated groups.

Biochemical findings

Values presented in Table 7 to14 indicate that the drug combination revealed significant effects on serum total protein, albumin, globulin, glutathione, malondialdehyde, glucose, sodium and potassium where, significant increasing (p<0.05) in serum total protein (g/dl) was recorded on the 6th week of the experiment (6.15± 0.29) and continued until the end of the 10th week (6.35± 0.11) with respect to different groups (Table 7).

Oxyclozanide and niclosamide combination caused significant increase (p<0.05) in blood albumin values (gm/dl) at the 6th week of the experiment (3.21 ± 0.16) and continued until the end of the 10th week (3.25 ± 0.12) with respect to different groups (Table 8). Significant increasing (p<0.05) in blood globulin values (gm/dl) were recorded on the 6th week of the experiment (2.94 ± 0.08) and continued until the end of the 10th week (3.10 ± 0.22) due to oxyclozanide and niclosamide combination with respect to different groups (Table 9). *Paramphistomes* infection caused significant decreasing (p<0.05) in serum glutathione values (µmol/g protein) from zero day, (13.76± 1.43) until the end of the experiment (14.73± 2.34). By treatment, these values were significantly increased (p<0.05) by the end of the experiment in other different groups (18.86± 1.72; 22.56± 3.12 and 20.74± 1.39, respectively) (Table 10).

Paramphistomes infection caused significant increase (p<0.05) in serum malondialdehyde levels (nmol /g protein) from zero day, (54.67± 1.52) until the end of the experiment (57.86± 2.54). By treatment, these values were significantly decreased (p<0.05) by the end of the experiment, especially in niclosamide and drug combination treated animals (34.86± 3.47 and 37.38± 2.24, respectively) (Table 11). Oxyclozanide, niclosamide and drug combination caused significant (p<0.05) improvement in serum glucose values (mg/dl) by the end of our trial (57.46± 2.73; 63.98± 3.26 and 69.47± 2.21, respectively) in comparison with the naturally infected non treated animals (52.98± 2.94) (Table 12). No statistical relevant differences in blood sodium values (mEq/L) remained within the same values between all groups (Table 13). No statistical relevant differences in blood potassium (mEq/L) remained within the same values between all groups (Table 14).

DISCUSSION

Paramphistomiasis is one of the most pathogenic diseases of domesticated animals, causing heavy losses to the livestock industry. It has been estimated that more than 500 million cattle worldwide are at risk due to parasitic infection (Juyal et al., 2003; Ilha et al., 2005). Clinical paramphistomiasis is usually diagnosed in cattle 4-18 months age as resistance developed after exposure to the parasite. This immunity protects the animal against the massive infections of immature fluke that causes such problems. Weaned cattle and lambs appear to be the most susceptible (Lloyd et al*.,* 2007).

It was confined to warmer tropical and subtropical areas of the world, and is associated with invasion of the duodenum and upper jejunum by large numbers of immature fluke (Rolfe and Boray, 1993; De Waal, 2010). Death due to immature *Paramphistomes* is very elevated and may reach to 80-90% in domesticated ruminants (Juyal et al*.,* 2003; Ilha et al*.,* 2005). *P. cervi* is considered to be one of the most important species of *Paramphistomes* since they are cattle parasites with a cosmopolitan distribution. The harm caused by the infection in bovine effects production, since these parameters provide a lower nutritious conversion, a loss of mass and a decrease in milk production, which causes economic losses (Ilha et al*.,* 2005).

Paramphistomiasis which is characterized by acute gastroenteritis occasionally occurred in cattle and buffaloes and rarely in sheep. Most infections of adult fluke are harmless although large numbers of fluke can cause a chronic ulcerative rumenitis with atrophy of rumenal papillae. Peak conical fluke numbers are usually seen in late summer or early winter following prolonged inundation of pasture (Smeal, 1995). Juvenile flukes attach to the intestinal mucosa. Catarrhal to necrotic and hemorrhagic duodenitis with less thickening may be seen in the early stages, progressing to be thickening (mucosal edema, submucosal hypertrophy), hemorrhages and ulceration. Anemia, hypoproteinemia (manifested as submandibular edema) and emaciation of the host ensue. After the juvenile fluke migrated to the rumen, the intestine repairs, leaving a thickened duodenum and jejunum as a result of diffuse mucosal and submucosal hypertrophy and fibrosis (Love and Hutchinson, 2003).

The hematological and biochemical findings revealed significant reduction in the total erythrocyte count, hemoglobin, packed cell volume, total protein, albumin, globulin and glucose. This could be attributed to the bloodsucking ability of parasites and hemorrhage that will lead to anemia as approximated to those reported by Gadre et al*.* (2008). Both sodium and potassium absorption occurred by distinct mechanisms in a major part of the intestine: in the jejunum, and potassium were mostly absorbed via co-transport, as a result of active uptake of sugars and amino acids; in the ileum, they were absorbed actively, against a significant electrochemical gradient. In the jejunum, sodium and potassium transports were greatly influenced by fluid movement and is stimulated by the presence of sugars; in the ileum, none of these

Table 7. Effect of orally administered of oxyclozanide (20 ml/100kg) or niclosamide (125 mg/kg) and both drugs on total protein (g/dl) of buffaloes naturally infected with *Paramphistomes* (n=5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	5.78±0.73^{bc}	5.78±0.15^{bc}	5.85±0.09^{bc}	5.88±0.26^{c}	5.91±0.34^{c}	5.96±0.18^{bc}
2nd group	5.88±0.42^{b}	5.91±0.24^{b}	5.94±0.24^{b}	5.95±0.18^{bc}	5.97±0.22^{bc}	5.99±0.24^{bc}
3rd group	5.80±0.23^{bc}	5.93±0.17^{b}	5.99±0.18^{b}	6.15±0.29^{a}	6.15±0.16^{a}	6.35±0.11^{a}
Control	5.45±0.73^{b}	5.48±0.03^{b}	5.62±0.16^{b}	5.80±0.22^{bc}	5.85±0.12^{bc}	5.48±0.13^{b}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

Table 8. Effect of orally administered of oxyclozanide (20 ml/100kg) or niclosamide (125 mg/kg) and both drugs on blood albumin values (gm/dl) of buffaloes naturally infected with *Paramphistomes* (n = 5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	3.00±0.65^{c}	3.03±0.15^{c}	3.04±0.09^{c}	3.06±0.14^{c}	3.04±0.14^{c}	3.05±0.05^{c}
2nd group	3.04±0.42^{c}	3.04±0.24^{c}	3.05±0.24^{c}	3.07±0.12^{c}	3.06±0.24^{c}	3.10±0.24^{b}
3rd group	3.00±0.23^{c}	3.09±0.25^{b}	3.12±0.29^{b}	3.21±0.16^{a}	3.20±0.15^{a}	3.25±0.12^{a}
Control	3.06±0.22^{c}	3.03±0.03^{c}	3.03±0.10^{c}	3.06±0.15^{c}	3.04±0.19^{c}	3.05±0.13^{c}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

Table 9. Effect of orally administered of oxyclozanide (20 ml/100 kg) or niclosamide (125 mg/kg) and both drugs on blood globulin values (gm/dl) of buffaloes naturally infected with *Paramphistomes* (n = 5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	2.78±0.12^{cd}	2. 75±0.14^{d}	2.81±0.11^{cd}	2.82±0.10^{c}	2.87±0.15^{c}	2.91±0.12^{b}
2nd group	2.84±0.14^{c}	2.87±0.18^{c}	2.87±0.19^{c}	2.88±0.12^{c}	2.91±0.08^{bc}	2.89±0.06^{bc}
3rd group	2.80±0.13^{cd}	2.84±0.17^{c}	2.87±0.14^{c}	2.94±0.08^{b}	2.95±0.11^{b}	3.10±0.22^{a}
Control	2.81±0.20^{c}	2.85±0.15^{c}	2.85±0.15^{c}	2.84±0.22^{c}	2.86±0.25^{c}	2.86±0.14^{c}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

Table 10. Effect of orally administered of oxyclozanide (20 ml/100 kg) or niclosamide (125 mg/kg) and both drugs on glutathione (µmol/g protein) of buffaloes naturally infected with *Paramphistomes* (n=5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	12.43± 2.31c	16.56± 3.25b	18.89± 2.44b	18.55± 1.53b	19.67± 2.47ab	18.86± 1.72b
2nd group	14.65± 3.43c	19.12± 2.64ab	19.21± 3.86ab	20.24± 3.55a	23.43± 2.68a	22.56± 3.12a
3rd group	11.43± 1.32c	16.62± 2.74b	17.42± 2.86b	18.67± 1.65b	19.43± 2.28ab	20.74± 1.39a
Control	13.76± 1.43c	13.87± 1.22c	12.45± 2.54c	13.23± 1.46c	13.37± 1.63c	14.73± 2.34c

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

factors affect sodium and potassium movement. They were also actively absorbed in the colon (Church, 1993). These facts could explain the non-statistically relevant differences in both blood sodium and potassium values

Table 11. Effect of orally administered of oxyclozanide (20 ml/100 kg) or niclosamide (125 mg/kg) and both drugs on malondialdehyde level (nmol /g protein) of buffaloes naturally infected with *Paramphistomes* (n=5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	54.88±3.42^{b}	62.88±2.33^{a}	61.88±2.52^{a}	57.88±1.67^{ab}	52.88±2.46^{b}	52.88±2.35^{b}
2nd group	51.27±3.46^{b}	40.27±3.57^{c}	37.27±2.35^{d}	36.27±3.52^{d}	34.27±1.52^{d}	34.86±3.47^{d}
3rd group	54.38±2.34^{b}	49.38±3.46^{bc}	46.38± 2.46^{c}	45.38±1.58^{c}	42.38±3.63^{c}	37.38±2.24^{c}
Control	54.67±1.52^{b}	58.98±2.42^{a}	55.76±1.76^{b}	59.87±1.73^{a}	57.98±2.41^{ab}	57.86±2.54^{ab}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

Table 12. Effect of orally administered of oxyclozanide (20 ml/100 kg) or niclosamide (125 mg/kg) and both drugs on serum glucose (mg/dl) of buffaloes naturally infected with *Paramphistomes* (n = 5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	53.13±2.43^{c}	52.34±2.55^{c}	49.62±2.23^{c}	56.58±2.47^{b}	58.97±2.33^{b}	57.46±2.73^{b}
2nd group	54.26±3.45^{c}	60.74±3.53^{ab}	61.63±3.72^{ab}	62.53±3.57^{ab}	64.16±3.73^{ab}	63.98±3.26^{ab}
3rd group	54.76±2.42^{c}	62.45±2.57^{ab}	63.30±2.32^{a}	62.52±2.64^{ab}	65.26±2.73^{a}	69.47±2.21^{a}
Control	54.57±3.52^{c}	54.08±3.43^{c}	54.67±3.35^{c}	55.39±3.46^{b}	57.94±3.53^{b}	52.98±2.94^{c}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

Table 13. Effect of orally administered of oxyclozanide (20 ml/100kg) or niclosamide (125 mg/kg) and both drugs on blood sodium values (mEq/L) of buffaloes naturally infected with *Paramphistomes* (n=5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	128.25±2.45^{ab}	129.40±6.12^{ab}	133.76±4.13^{a}	135.46±4.56^{a}	136.47±4.55^{a}	137±4.22^{a}
2nd group	129.45±4.16^{ab}	134.56±2.45^{a}	136.22±5.13^{a}	135.13±5.10^{a}	138.12±7.35^{a}	136.16±6.43^{a}
3rd group	130.16±4.76^{ab}	133.24±7.42^{a}	135.44±3.12^{a}	135.76±3.45^{a}	134.25±6.12^{a}	135.22±3.36^{a}
Control	134.14±4.12^{a}	135±2.45^{a}	135.10±5.13^{a}	137.20±2.46^{a}	136.14±5.43^{a}	134.24±5.42^{a}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

Table 14. Effect of orally administered of oxyclozanide (20 ml/100 kg) or niclosamide (125 mg/kg) and both drugs on values of blood potassium (mEq/L) of buffaloes naturally infected with *Paramphistomes* (n=5).

Weeks / Groups	0 day	2nd week	4th week	6th week	8th week	10th week
1st group	4.04±0.7^{c}	4.20±0.14^{b}	4.18±0.14^{b}	4.43±0.21^{a}	4.52±0.15^{b}	4.44±0.14^{a}
2nd group	4.02±0.05^{c}	4.16±0.16^{b}	4.24±0.11^{b}	4.44±0.11^{a}	4.65±0.05^{b}	4.66±0.14^{a}
3rd group	4.00±0.16^{c}	4.22±0.14^{b}	4.28±0.17^{b}	4.38±0.07^{ab}	4.35±0.18^{ab}	4.37±0.04^{ab}
Control	4.05±0.12^{c}	4.10±0.11^{bc}	4.20±0.04^{b}	4.45±0.07^{a}	4.42±0.13^{a}	4.46±0.21^{a}

Values are expressed as Mean ± SE. The means which carry different letters in the same column were significantly different, ($p < 0.05$).

that remained within the same values between all groups in our trial.

The high intracellular content of glutathione (GSH) in the liver is congruous with the detoxification functions of

this organ. Regular dietary intake of precursor sulfur containing amino acids will maintain hepatic intracellular GSH levels in the 5–10 mM range. Alterations in liver GSH are either the cause or the effect of a number of pathologies (Townsend et al*.,* 2003). So, absorption impairment by intestinal diseases (paramphistomiasis) to sulfur containing amino acids (like: cyst(e)ine) could decrease liver production of glutathione that approximated in our findings.

Furthermore, intestinal affection could decrease the activity of key enzymes (gamma-glutamylcysteine synthetase and gamma-glutamyl transferase) involved in GSH synthesis accompanied by a decreased availability of cyst(e)ine for GSH synthesis contribute to mucosal GSH deficiency in IBD. As the impaired mucosal antioxidative capacity may further promote oxidative damage, promoted reactive oxygen species contribute to tissue injury through polyunsaturated fats can readily undergo peroxidation to yield lipid hydroperoxides that are potentially toxic to the intestine and other tissues when absorbed from the lumen (Sido et al*.,* 1998; Serdar et al*.,* 2008).

That could explain the decreased body weight gain in naturally infected non treated buffaloes beside the previously mentioned effect of the parasite on the absorption of nutrient from the intestine.

During the tissue invasion by immature stages of *Paramphistomum* spp., it is exposed to elevated amounts of exogenous reactive oxygen species (ROS), such as superoxide radical anions (O_2^-) and hydrogen peroxide (H_2O_2). Additionally, the parasite can stimulate the activation of the host's immune response, which results in production of the cytokines TNF-α and IFN- α, and these increase respiratory burst (and ROS) on phagocytes. These highly toxic molecules cause severe damage to biological macromolecules (such as lipids, proteins and DNA) leading to metabolic malfunctions. Lipid peroxidation reflects the interaction between ROS and polyunsaturated fatty acids, and induces oxidation of various breakdown products of the latter. Among these, malondialdehyde (MDA) is a reliable marker of oxidative damage (Sido et al*.,* 1998; Serdar et al*.,* 2008).

As a result, lipid peroxidation causes changes in membrane permeability and selectivity and ultimately leads to alterations in cell volume homeostasis and cellular metabolism. Moreover, hydroperoxides and aldehydes are directly toxic to cells and organelles (Namıduru et al., 2011). So, the obtained data suggested that reactive oxygen metabolites mediate injury is important in both primary and downstream secondary pathophysiological mechanisms underlying intestinal inflammation. At the same time, these parameters (glutathione and malondialdehyde) could be of importance to be used as a supplement the conventional microscopic method for reliable diagnostic method of intestinal parasitism especially in case of the parasite not revealed by examination of a single fecal sample. Periodic medication will not only help in the prevention of outbreaks of acute paramphistomiasis but also in preventing fecal contamination of environments by reducing egg output, thus interrupting the life cycle of the parasite. In view of this, our trial was begun with evaluation of the combination of oxyclozanide and niclosamide.

Oxyclozanide [2,3,5-trichloro-N-(3,5-dichloro-2-hydroxyphenyl)-6-hydroxybenzamide] was a salicylanilide anthelmintic that mainly acts by uncoupling oxidative phosphorylation in flukes (Jo et al*.,* 2011). Early investigations were carried on oxyclozanide in mice. They were infected by gavage with one or two metacercariae treated over different periods covering the whole of the immature phase of migration of *Fasciola hepatica* and then killed either 21 or 31 days after infection. Only the examined drug was generally ineffective over -1 to six days after infection, and displayed varying activity over the other periods. Flukes recovered from the treated animals were retarded in size and there were corresponding reductions in liver pathology. Oxyclozanide had little effect on 21-day-old flukes at 0.1% in the diet but had 100% efficacy at concentrations of 0.2 and 0.5%. The 0.5% level was 88% effective at 7 and 14-day-old infections (Probert et al*.,* 1981). Oxyclozanide was effective against adult liver flukes (*F. hepatica*), but only partially against later immature stages (Coles and Stafford, 2001). After oral administration, oxyclozanide was found mainly in liver, kidneys and gut. It was slowly metabolized and excreted through the bile and the feces (Jo et al*.,* 2011).

Both oxyclozanide and niclosamide belong to salicylanilides. The molecular mode of action of salicylanilides is not completely elucidated. They all are uncouplers of the oxidative phosphorylation in the cell mitochondria; inhibit the coupling between the electron transport and phosphorylation reactions and thus inhibit ATP synthesis, the cellular "fuel." This impairs the parasite's motility and probably other processes as well. Niclosamide acts on the tapeworms also through inhibition of glucose absorption (Terada, 1990 ; Mehlhorn, 2008).

The chemotherapeutic value against rumen flukes (*Paramphistomum* spp) could be limited and also evident in this study, the significant continuation of eggs shedding and partial low drug efficacy (%) all over the experimental period could be attributed to the survival of immature stages, and so they are capable of being mature as a second wave of infection. Another possible mechanism of survival suggested that the drug could cause incomplete atrophy to the generative tissues (gonads) and this depend upon the physiological status of the parasite (decreasing the energy caused by oxyclozanide) as mentioned before by Stammers (1975). Another data recorded by Rolfe and Boray (1987) mentioned that oxyclozanide at 18.7 mg/kg reduced parasite (*Calicophoron calicophorum*) numbers in the small intestine, abomasum and rumen-reticulum by 61 to 96.1%, 50.0 to 92.6% and 56.5 to 98.1%, respectively. When 2 doses were given 3 days apart, oxyclozanide was 99.9%, 100 and 100% effective, correspondingly, in the above organs, and produced

improvement in clinically affected calves. This disagreement with our mentioned data could be attributed to species of examining parasites.

Furthermore, the limited efficacy of oxyclozanide was approximated by the significant reduction in red blood cell count, hemoglobin, packed cell volume, total protein, blood albumin, blood globulin, glutathione, malondialdehyde, and serum glucose which began from the 6th week of the experiment to the end of the trial. Finally, these findings reflected on the body weight gain which significantly decreased when compared with the drug combination group of buffaloes naturally infected with *Paramphistomes*.

Niclosamide was poorly absorbed in the gut and was excreted through the feces almost completely as the unchanged parent compound (Saeb-Parsy et al*.,* 1999). Niclosamide was effective for immature tapeworms (for example, *Taenia*, *Moniezia* spp), and against several blood flukes (*Schistosoma* spp.). It was originally introduced as a molluscicide, that is, a snail killer, to control those snails that transmit schistosomiasis (Mehlhorn, 2001). The earlier study was conducted by Rolfe and Boray (1987). They controlled tests to assess the efficacy of anthelmintics against immature *Paramphistomes*, predominantly *Calicophoron calicophorum*, in 127 calves, which were exposed to contaminated pasture for seven weeks, treated and slaughtered. Niclosamide efficacy at 160 mg/kg given as single or two doses three days apart were 91.1 and 92.6% effective, respectively, against the parasites in the small intestine (the immature stages of *Paramphistomum* spp.).

Based on fecal egg count data, the continuation and nearly constant rate of egg production clearly demonstrated that niclosamide could effect on the immature stages in the intestine rather than the mature worms that maintain egg production. As niclosamide is poorly absorbed in the gut, so the drug was difficultly accumulated around the developed worms to be ingested by the parasite and could affect it like soluble form or the drug is more rapidly absorbed when worms are contacted in the earlier times of their lives (Kumchoo et al*.,* 2007). Rangel-Ruiz et al*.* (2003) mentioned that adult *Paramphistomes* attached to the villi in the rumens of definitive ruminant hosts and feed on nutrients from the rumens, omasums and abomasums, causing weight loss and a decrease in milk production although they can wander into the bile and pancreatic ducts, as do other trematodes.

Depending on these data, our trial clearly demonstrated that the limited efficacy of niclosamide effect on immature stages of *Paramphistomum spp*., was approximated by the relevant reduction in red blood cell count, hemoglobin, packed cell volume, total protein, blood albumin, blood globulin, glutathione, malondialdehyde, and serum glucose which began from the 6th week of the experiment to the end of the trial. Significant continuation of egg shedding and sharp low drug efficacy (%) all over the experimental period could be attributed to the survival of mature stages, and so they are capable of egg production and affecting animal health as well. Finally, these findings reflected in the body weight gained that significantly decreased when compared with the drug combination group of buffaloes naturally infected with *Paramphistomes*.

Oxyclozanide and niclosamide combination in the recommended dosage regimen is considered of choice for the treatment of *Paramphistomum* infection in buffaloes. In the present trial, the obtained data showed that, this combination significantly reduced number of egg count and improved drug efficacy (%), hematological and biochemical profiles. Besides, this combination can overcome the oxidative stress due to the infection by improving GSH concentration that gradually increased and lowering the level of malondialdehyde level in serum at the end of the trial. Finally, we could concluded that, treatment of infected animals with the tested drugs (oxyclozanide and niclosamide) resulted in amelioration of the most adverse effects associated with this infection, decreased egg count and oxidative stress, improved biochemical and hematological profiles.

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