THE INFLUENCE OF NITROXYNIL ON CLINICAL, BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN EGYPTIAN BUFFALOES AFFECTED WITH LIVER FLUKE

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Abstract

The present investigation was carried out to clarify the hematological and serum biochemical changes associated with hepatic fascioliasis in Egyptian buffaloes. Two hundred and fifty buffaloes (2-6 years old) from different localities at Sharkia Governorate were examined clinically and parasitologically. Eighty buffaloes out of all examined ones (32.0%) had been infected with Fasciola spp. Prevalance of parasitic infestation (42%) was found in winter while, the lowest infestation rate was found during summer season (22%). Infected animals milk yield, watery foetid diarrhea and dehydration were recorded. Infected buffaloes with hepatic fascioliasis showed (normocytic normochromic anemia), leukocytosis with neutropenia, lymphocytosis and eosinophilia. Serum biochemical changes revealed hypoproteinaemia, hypoalbuminaemia, hyperglobulinaemia with decrease of A/G ratio, also, a significant decrease of glucose, iron, copper, zinc, calcium, inorganic phosphorus, magnesium and potassium. There were highly significant increases of sodium, total bilirubin and enzymatic activities of serum aspartate aminotransferase (S.AST), serum alanine aminotransferase (S.ALT), serum alkaline phosphatase (S.AP) and serum lactate dehydrogenase enzyme (S.LDH). Moreover, all blood parameters progressed to their normal levels with the progress towards recovery after treatment with "fasciolid" anthelmintic drug. The public health hazard effects were discusseds and suggestive recommendations were recorded.

INTRODUCTION

Bovine fascioliasis is one of the most important trematode parasitic diseases which exists allover the world due to the presence of the suitable environments for the intermediate host snails. Fascioliasis causes liver damage and consequently the metabolic processes of liver are gradually reduced (Fikry *et al.* 1988). Liver fluke

the intermediate host snails. Fascioliasis causes liver damage and consequently the metabolic processes of liver are gradually reduced (Fikry *et al.* 1988). Liver fluke varies greatly between countries and regions depending on climatic conditions and the management behaviour where parasitic infestation is usually associated with the economic losses in buffalo meat, milk and fertility (Nabila *et al.* 1990). Penny *et al.* (1996) reported anaemia in buffaloes infested with *Fasciola hepatica* and followed by death when hemoglobin content dropped to 4 g%. The author recorded also that newborn calves from buffaloes affected with fascioliasis showed lowered carcass weight than calves parturated from non-fasciola infested dams.

Pica (eating of earth) was due to metabolic disorders in serum proteins, minerals and trace elements. Jones *et al.* (1997) concluded that in domestic ruminants with fascioliasis, there was a positive correlation between liver fluke numbers and serum, potassium, calcium, phosphorus and glucose. In addition, Mohamed (2000) and Omran *et al.* (2000) reported normocytic normochromic anaemia, leukopenea, lymphopenia and eosinophilia in Egyptian buffaloes with fascioliasis. Amer *et al.* (2002) and Radostits *et al.* (2002) recorded that the economic losses due to metabolic disorders in diseased buffaloes were 30 times higher than losses due to moralities or condemned liver organs in the abattoirs.

Therefore, it was of great importance to study the problem of fascioliasis in Egyptian buffaloes in Sharkia province with regarding to its rate, seasonality of the disease as well as the pathognomonic effect through clinical, hematological and serum biochemical changes. In addition, the flukicidal effect of fasciolid anthelmintic drug was assessed.

MATERIALS AND METHODS

Animals

Two hundred and fifty buffaloes of different ages (2-6 years old) belonging to private farms in different localities at Sharkia province were examined clinically and parasitologically. These animals showed clinical signs of emaciation, icteric and paleness mucous membranes, intermittent tympany followed with diarrhoea in most of them while, there was a pica and submandibular oedema in others.

1- Fecal samples

Fecal samples were collected directly from the rectum of all animals (n = 250), prepared by sedimentation technique and examined microscopically for detection of parasitic ova (Kelly, 1984). Accordingly, the examined animals were divided into two groups. The first group consisted of ten clinically and parasitologically healthy buffaloes used as controls. The second group consisted of eighty heavily infected buffaloes with liver fluke.

Fecal samples and blood samples were collected from heavily infected buffaloes (n = 80) before and two weeks after treatment with fasciolid drug (nitroxynil 25% solution, Chemical industries development, CID Pharmachem Bulgaria) by subcutaneous route of injection at a dose level of 1 ml/25 kg body weight. The eggs per gram of faeces were determined according to Anderson *et al.* (1993).

2- Blood samples

Two jugular blood samples were collected from heavily infected buffaloes (n = 80) before and two weeks after treatment as well as from ten clinically and parasitologically healthy buffaloes used as control group. The first blood sample was taken in heparinized vacutainer tube for hematological studies of erythrocytic count (RBCs), hemoglobin concentration (Hb%), and packed cell volume (PCV%). Erythrocytic indices were performed mathematically "Mean corpuscular hemoglobin" (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), as well as leucocytic counts and their differentiation using the method described by Coles, (1986). The second blood sample was taken into clean dry centrifuge tubes, left to clot, then centrifuged at 3000 r.p.m for 20 minutes and the separated clear serum was used for determination of total serum protein (Kingsley, 1972), and albumin (Doumas *et al.* 1971).

Determination of total serum bilirubin (Jendrassik and Grof, 1938), serum glucose (Trinder, 1969), as well as, determination of serum enzymatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to Reitman and Frankel (1957) were carried out . Serum alkaline phosphatase (SAP) according to Kind and King method (1954) and lactic dehydrogenase enzyme (LDH)

according to King (1969). Serum calicium, inorganic phosphorus, sodium, potassium, copper, zinc and iron were estimated by using atomic absorption spectrophotometer (PYE-Unicum, Sp-90, England) according to Allain and Mauros, (1979).

Statistical analysis of the data was performed according to Snedecor and Cochran (1982).

RESULTS

Eighty buffaloes out of all examined ones (25%) (representing 32.0%) were found to be infested with fascioliasis in this study. General weakness, pale mucous membranes, indigestion, as well as, recurrent tympany and intermittent diarrhea were noticed in some of them. Intermandibular edema, decrease in milk yield and pica were the most recorded clinical symptoms. The high infection rate was recorded in winter season (41.24%), while, the lowest level was recorded in summer (16.0%), in spring and autumn as the incidences were (33.33%) and (31.66%), respectively, (Table, 1). In a trial for treatment, which was judged by fecal eggs count before and two week after treatment, fasciolid proved to be highly effective against Fasciola where egg counts were reduced to 95.0% (Table, 2) as the mean values of blood picture and serum parameters showed an improvement towards the normal levels after treatment. The results of hematological and serum biochemical changes before and after treatment were shown in Tables 3 and 4).

Table 1. Seasonal incidence of Fasciola infestation among buffaloes before and after treatment with nitroxynil.

Season	No. of examined animals	No. of positive animals	Percent of infestation	Mean No. of eggs/gm of feces	
				Before treat.	*After treat.*
Winter	80	33.0	41.24%	209 ± 15	8 ± 3.0
Spring	60	20.0	33.33%	130 ± 12	7 ± 2.0
Summer	50	8.0	16.0%	75.0 ± 17	6.0 ± 1.0
Autumn	60	19.0	31.66%	160.0 ± 16	8.0 ± 2.0
Total	250	80.0	32.0%	143.5 ± 15	7.25 ± 2.0

Table 2. Anthelmintic efficacy of fasciolid drug Nitroxynil against fascioliasis in buffaloes in Sharkia Governorate.

Drug	Dose	Rout of administration	No. of eggs/gm of feces before treatment	No. of eggs/gm of feces after treatment	Efficacy%
Fasciolid (nitroxynil 25%)	1 ml/25kg	S/C	143.5 ± 15.0	7.25 ± 2.0	95%

Table 3. Hematological values of healthy and diseased buffaloes with fascioliasis before and after treatment with nitroxynil.

Parameters	Unit	Healthy buffaloes (n = 10)	Diseased buffaloes with fascioliasis (n = 80)	
			Before treatment	*After treatment*
RBCs	10^6/ul	8.62 ± 0.25	6.00 ± 0.27**	8.22 ± 0.15
Hb	g/dl	11.22 ± 0.23	9.50 ± 0.33**	10.95 ± 0.25
PCV	%	35.11± 2.12	28.00 ± 0.98**	33.75 ± 1.92
MCV	Fl	41.95 ± 1.5	44.75 ± 1.62	41.22 ± 1.23
MCH	Pg	15.35 ± 0.75	18.30 ± 0.25	14.27 ± 0.75
MCHC	%	35.22 ± 1.22	36.25 ± 1.21	35.77 ± 0.99
Total WBCs	10^3/ul	13.22 ± 0.75	19.0 ± 0.95**	13.21 ± 0.55**
Neutrophils	%	55.0	43.0**	52.0**
Lymphocytes	%	37.0	45.0**	40.0**
Eosinophils	%	2.5	5.50*	3.00**
Basophils	%	1.5	0.90	1.40
Monocytes	%	3.9	3.60(N.S)	3.60

* Significant at ($P < 0.05$).

** Highly significant at ($P < 0.01$).

(N.S) Non significant at ($P > 0.05$).

Table 4. Biochemical changes of healthy and Fasciola infested buffaloes before and after treatment with nitroxynil .

Group / Parameter	Clinically healthy (control)	Diseased buffaloes	
		Before treatment	*After treatment*
Total protein (g/dl)	7.86 ± 0.64	6.50 ± 0.24**	7.25 ± 0.20*
Albumin (g/dl)	3.98 ± 0.15	2.03 ± 0.10**	3.45 ± 0.15
Globulin (g/dl)	3.88 ± 0.49	4.47 ± 0.14**	3.80 ± 0.05
A/G ratio	1.04 ± 0.05	0.83 ± 0.04*	0.95 ± 0.03
Creatinine (mg/dl)	1.99 ± 0.12	1.88 ± 0.25 (N.S)	1.79 ± 0.13
Total bilirubin (mg/dl)	0.26 ± 0.06	0.26 ± 0.06**	0.25 ± 0.05
Glucose (mg/dl)	81.0 ± 2.25	61.0 ± 6.27**	79.0 ± 2.15
Sodium (mE q/L)	141.0 ± 4.55	155.0 ± 8.75*	139.0 ± 4.12
Potassium (mE q/L)	7.22 ± 0.75	4.75 ± 0.66*	6.98 ± 0.88
Na/k ratio.	20.11± 0.05	38.75±0.25	23.18±0.17
Copper (ug/100 ml)	129.0 ± 6.22	98.0 ± 9.22**	123.0 ± 8.78
Zinc (ug/100 ml)	99.0 ± 5.78	69.0 ± 7.25**	91.0 ± 7.62
Iron (ug/100 ml)	168.0 ± 8.99	82.0 ± 5.25**	163 ± 6.27
Calcium (mg/dl)	11.8 ± 0.88	8.15 ± 0.17**	10.92 ± 0.55
Inorganic phosphorous (mg/dl)	7.1 ± 0.25	4.75 ± 0.27**	6.85 ± 0.32
Magnesium (mg/dl)	2.5 ± 0.12	1.6 ± 0.05**	2.3 ± 0.11
S.AST (IU/L)	75.0 ± 5.25	112.0 ± 10.25**	71.0 ± 6.22
S.ALT (IU/L)	15.0 ± 3.22	25.0 ± 5.75**	13.0 ± 2.15
S.AP (IU/L)	91.25 ± 7.72	240.0 ± 25.82**	89.0 ± 8.75
S.LDH (IU/L)	315.0 ± 44.0	309.0 ± 55.0 (N.S)	312.0 ± 49.0

* Significant at ($P < 0.05$).

** Highly significant at ($P < 0.01$).

(N.S) Non significant at ($P > 0.05$).

DISCUSSION

The present investigation revealed that the incidence of fascioliasis in buffaloes in Sharkia province was very high representing 32.0%. This is governed mainly by the population dynamics of the intermediate host snails which in turn are affected greatly by the prevailing climatic pattern through its effect on vegetation. The highest

infection rate was recorded in winter (41.24%) and the lowest infection rate was recorded in summer (16.0%) while, it was (33.33%) and (31.66%) in spring and autumn, respectively, (Table, 1). These variations have been reported by Mohamed (2000) and may be attributed to the effect of the climatic conditions on hatchability of Fasciola eggs which may provide a favourable condition for rapid and persistent contamination of the surrounding environments.

The obtained clinical symptoms in some buffaloes were emaciation, poor body conditions, depression, dullness, roughness of coat and diarrhea. Other buffaloes showed paleness and icteric appearances of the mucous membranes, recurrent ruminal stasis, submandibular oedema and decreased milk production. The obtained clinical signs were similar to those previously reported by Fikry *et al.* (1988). Nwiyi & Chaudrai (1996) and Abdel-Salam *et al.* (1998). Such signs appear only with heavy infestation for long period as a result of severe damage of liver tissues.

Hematological studies recorded in Table 3 indicated that the diseased buffaloes have a normocytic normochromic anemia. A highly significant decrease ($P < 0.01$) in erythrocytic count, hemoglobin content and packed cell volume with marked leucocytosis were also recorded. These could be attributed to the severe anaemic condition which may be due to inability to assimilate the hemopoietic principles or due to a chronic liver inflammation which causes depression of erythrogenesis (Coles, 1986). On the other hand, leucocytosis with neutropenia lymphocytosis and eosinophilia were obtained as means of defence of the body against Fasciola destructive effects or due to toxin mediated lesion of the bone marrow (Penny *et al.* 1996). These results coincided with those previously recorded by Pandy *et al.* (1995).

The results of biochemical analysis revealed evidence of highly significant decrease ($P < 0.01$) of total serum protein and albumin in blood of buffaloes affected with fascioliasis as recorded in Table 4. This may be attributed to the damaged liver and poor absorption of the nutrients. Coles (1986), Jones *et al.* (1997) and Mohamed (2000) gave another explaination that in buffaloes with chronic fascioliasis, liver cirrhosis showed significant hypoproteinaemia, hypoalbuminaemia and reduction of A/G ratio because of the severe infestation of the liver with Fasciola spp. Severe destruction of the liver parenchyma resulted in rise to drastic alterations in plasma

protein values and this may be observed in association with liver diseases. The authors added that decrease in the albumin level may be due to inhibition of albumin synthesis or an increased concentration of globulin which usually may indicate rapid breakdown or loss of albumin. Regarding to total globulin (Table 4) a highly significant change ($P < 0.01$) was observed in the serum of parasitized buffaloes. The obtained results were similar to those previously reported by Abdel-Salam *et al.* (1998) and Amer *et al.* (2002).

Concerning the effect of fascioliasis on serum creatinine value in buffaloes, the mean value of serum creatinine in normal buffaloes was (1.99 ± 0.12 mg%) while, in diseased buffaloes was (1.81 ± 0.15 mg%) which remained unchanged in Fasciola infested animals. It is evident that fascioliasis had no deleterious effect on the muscle fibers as destruction of the latter is associated with raised creatinine level (Fikry *et al.*, 1988, and Abdel-Salam *et al.*, 1998).

The results of biochemical analysis revealed evidence of highly significant increase ($P < 0.01$) of total serum bilirubin (Table 4). These increases may be due to hepatic jaundice, which is associated with the hepatic and extrahepatic damage as well as disturbances in the efficiency of bile excretion. These results were in agreement with those reported by Nabila *et al.* (1990) and Amer *et al.* (2002). Our results revealed hypocalcaemia, hypomagnesaemia, hypophosphtemia and hypoglycaemia (Table, 4) which may be due to the degree of alterations in liver function under this type of parasitism.. Similar findings were reported by Fikry *et al.* (1988), Penny *et al.* (1996), and Radostits *et al.* (2002). The authors gave another attribution, where the presence of adult Fasciola in the bile ducts may interfere with the bile secretion or its passage into the intestine causing disturbances in the absorption and utilization of dietary calcium, magnesium, phosphorus and glucose. Table 4 showed a significant increase in sodium and significant decrease of potassium (Table 3), which however returned to its normal levels after treatment. The observed hypernatraemia and hypokalaemia could be attributed to the hepatic pathological conditions and diffuse cell death associated with liver affection. There was a highly significant decrease ($P < 0.01$) of serum iron, copper and zinc (Table, 3) in blood of buffaloes naturally infested with Fasciola and this pointed out to the association of disturbed mineral metabolism with fascolasis. The effect of fasciolasis in buffaloes as shown in Table 3 indicated a highly significant increase ($P < 0.01$) in serum AST, ALT and AP while, there was no-significant change in serum LDH enzymatic activities due to the degenerative changes and cirrhosis of the liver tissues as well as the enlargement of the gall bladder in which the adult worms could be

observed. Our results are in agreement with those of Amer *et al.* (2002) and Radostits *et al.* (2002).

The flukcidal effect of (Nitroxynil 25%) against natural fascioliasis in buffaloes was determined on the basis of fecal egg counts before and two weeks after treatment (Table 2). Nitroxynil 25% was highly effective against adult Fasciola worms if used subcutaneously at a dose of 1ml/25 kg body weight in buffaloes. However, some eggs appeared in fecal samples of some cases after two weeks from treatment and disappeared after that. This may be attributed to the discharging of remaining eggs in the gall bladder. Furthermore, regarding to the significantly affected parameters of the hematological and serum biochemical studies of diseased buffaloes two weeks after treatment were enhanced towards the values of the controls. Therefore, the examination of fecal samples in buffalo flucks at regular intervals reflect either regression or progression of respective diseased conditions and illustrating the efficacy of therapeutic trials.

It could be concluded that there was a correlation between the changes in blood constituents and clinical signs in buffaloes affected with liver fluke. Also, it could be concluded that the serious effect of fascioliasis has been subjected of controversy and has not been fully flukcidated and further information would be needed on its epidemiology in other provinces of Egypt. Neverthless, the veterinarians should take a much closer look at the beginning of the life cycle of Fasciola spp. and try to consistently eliminate the excretion of eggs and the following infection of the intermediate hosts by carefully planning microscopical surveys and taking chemotherapeutical measures. On the long run, this way only will be possible to gain control over liver fluke infection in humans.

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دراسات علي تاثير عقار النيتروكسينيل علي بعض التغيرات الدموية والبيوكيميائية في الجاموس المصري المصاب بالديدان الكبدية في محافظة الشرقية

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أجريت هذه الدراسة علي عدد ٢٥٠ حالة من الجاموس المصري تتراوح أعمارها من ٢-٦ سنوات من مزارع خاصة في مناطق مختلفة في محافظة الشرقية وكانت هذه الحالات تعاني من ضعف عام وهزال وعدم الشهية للأكل وقلة إنتاج الألبان – إسهال ونفاخ متكرر والأغشية المخاطية باهتة اللون وأوديما بين الفكين في بعض الجاموس. تم أخذ عينات براز من جميع هذه الحيوانات وبالفحص المعملي الطفيلي بفحص عدد بويضات الديدان الكبدية في البراز لكل حيوان وجد أن ٨٠ جاموسة مصابة بالديدان الكبدية بنسبة ٣٢% من إجمالي الحيوانات التي فحصت. سجلت أعلي قيم للإصابة في فصل الشتاء (٤١,٢٤%) ثم فصل الربيع (٣٣,٣٣%) وفي الخريف (٣١,٦٦%) أما الصيف فكانت أقل قيم الإصابة (١٦%).

تم أخذ عينتين من دم وسيرم كل حيوان من جميع الحيوانات المريضة (٨٠ جاموسة) والسليمة الضابطة (١٠ جاموسة) لمعرفة تأثير الإصابة بالديدان الكبدية علي مكونات الدم والسيرم بالنسبة لصورة الدم أظهرت النتائج وجود أنيميا ذات الخلايا الطبيعية الحجم والشكل مع انخفاض في مستوى كل من عدد كرات الدم الحمراء والهيموجلوبين وحجم خلايا الدم المضغوطة بينما كان هناك زيادة في عدد كرات الدم البيضاء وأظهرت النتائج البيوكيميائية قبل العلاج نقصاً معنوياً في عناصر البروتين الكلي والألبيومين والجلكوز والكالسيوم والفوسفور والماغنسيوم والبوتاسيوم والحديد والنحاس والزنك بينما وجد زيادة معنوية عالية في مستوى عنصر الصوديوم والمجموع الكلي للأصباغ المرارية وكذلك ارتفاع النشاط الأنزيمي في الدم (S.AST, S.ALT, S.AP and S.LDH) أما الجلوبيولين والكرياتينين قد تغير تغيراً غير معنوى . وقد تم علاج الجاموس المصاب بحقن عقار الفاشيوليد ١سم/٢٥كجم من وزن الحيوان تحت الجلد. وبعد العلاج تم أخذ عينات براز ودم وسيرم مرة أخرى لمعرفة نتائج العلاج طفيليا وبيوكيميائيا. وقد أوضحت النتائج استجابة الجاموس المصاب للعلاج وكان معدل نقص اختزال البويضات بدرجة ٩٥% وبعد أسبوعين من العلاج اختفت تلك البويضات نهائيا مما أدي إلي اختفاء الأعراض المرضية تدريجيا. لوحظ تحسناً ملحوظا في مكونات الدم والسيرم حتى تقاربت مع المعدلات الطبيعية. من هذه الدراسة يتضح أن الإصابة بهذا الطفيل الخطير يؤدي إلي تدمير مكونات الدم والسيرم وبالتالي إلي خسارة اقتصادية كبيرة في اللحوم والألبان والثروة الحيوانية والوقاية منه مبكراً يمنع وصوله إلي الإنسان وتدميره كذلك.