SOME HEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS CONSEQUENT TO CONCURRENT ADMINISTRATION OF IVERMECTIN AND RAFOXANIDE IN RAMS

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ABSTRACT

Ivermectin and Rafoxanide are widely used in sheep as endoectoparasiticidal and fasciolicidal drugs. The effects of ivermectin and rafoxanide as well as their concurrent use as prophylactic drugs on hematological and serum biochemical parameters were studied in 20 apparently clinically healthy rams, classified into 4 equal groups. The 1st group was left without treatment (control). The 2nd group was injected s/c with ivermectin (0.2 mg kg⁻¹). The 3rd group was injected s/c with rafoxanide (3 mg kg⁻¹). While, the 4th group was injected s/c with both drugs (0.2 mg ivermectin and 3 mg rafoxanide kg⁻¹). Blood samples were collected weekly for 2 weeks for hematological and biochemical studies. The obtained results with ivermectin treated group revealed, a significant decrease in erythrocytic count, PCV % and Hb concentration, MCV and MCHC%. Meanwhile, significant increase in serum AST and ALT activities was recorded. The rafoxanide treated group provoked a significant decrease in leuckocytic and erythrocytic counts, Hb%, PCV%, MCV, MCH and MCHC% as well as serum triglycerides level. Meanwhile, a significant increase in serum cholesterol and LDL-c levels was reported. Moreover, the group treated S/C with both drugs, elicited a significant decrease in erythrocytic and leukocytic
counts, PCV%, MCV, MCH, MCHC%, triglyceride levels and Hb%. In contrast, a significant increase in AST, ALT activities, cholesterol and LDL-c levels was recorded. While, in comparison with ivermectin and rafoxanide treated groups, there was insignificant changes in albumin, blood urea nitrogen, creatinine, HDL-c and erythrocytic count. In opposite side, there was a significant increase in serum AST, ALT and LDL-c levels as compared with ivermectin and rafoxanide treated groups. Also, there was a significant decrease in Hb%, PCV%, MCV, MCH and MCHC%. Meanwhile, a significant increase in serum cholesterol level as compared with ivermectin treated group. Moreover, there was insignificant changes in serum cholesterol and triglyceride levels, erythrocytic count, Hb%, PCV%, MCH and MCHC% as compared with rafoxanide treated group. In conclusion it could be concluded that hematological and biochemical alterations obtained with concurrent administration of both ivermectin and rafoxanide didn’t differ greatly than those obtained with each of them when injected alone and there is no additional dangerous from this combination.

INTRODUCTION

Anthelmintics are widely used for combating or treating different helminthes infections of different animal species. Chemotherapy of parasitic infestations with a wide spectrum of activity and minimal side effects are the main objects. Ivermectin, a potent broad spectrum antiparasitic drug from macrocyclic lactone family (Lespine et al., 2006) is a mixture of two natural fermentation products of Streptomyces avermitilis. It is a 22,23-dihydroivermectin B$_{1a}$ and is usually marketed as a mixture of 22,23-dihydroivermectin B$_{1a}$(> 80%) and 22,23-dihydroivermectin B$_{1b}$ (Mackellar and Benchaoui, 1996). The drug
exerts its toxic action by blocking the excitatory motor neurons in parasites which contain Gamma Amino Butyric Acid (GABA) in their nervous system (Leaning, 1981 and Barragry, 1987). Consequently, nematodes and arthropods that rely on GABA-mediated neural transmission are affected, while cestodes and trematodes which do not rely on GABA-mediated neural transmission are `not affected (Egerton et al., 1980 and Campbell et al., 1963) Moreover; the drug has ovicidal as well as larvicidal effects (Anantaphruti et al., 1982). In sheep and goats ivermectin is highly effective against all the important pathogenic gastrointestinal nematodes, lung nematodes, nasal bots and mites (Mackellar and Benchaoui, 1996) nevertheless, the extremely potent antiparasitic activity of ivermectin is greatly averted by its frequently encountered adverse effects. Detailed studies divulged hepatitis in goats (Ali and Abu-Samra, 1987) and altered hepatic function in rams (Hassan et al., 1995) in response to ivermectin treatment. The previous side effects could represent a crimp in substantially efficient use of this drug in these species. Rafoxanide is a member of halogenated alicylanilides with potent antiparasitic activity. It has the chemical formula [3′-chloro-4′ (p-chlorophenoxy)-3, 5 diiodosalicylanilide] (Adams, 1995). It is a widely used in sheep as a fasciolicidal drug. It is used for the control of Fasciola spp, Haemonchus spp (Swan, 1999) and Oestus ovis (Sanyal et al., 1986 and Swan, 1999) infestations in sheep in many parts of the world. Its fasciolicidal effect in sheep depends on the persistence of the drug in plasma. It is extensively bound to plasma protein and has a long half life about (16.6 day) as reported by Barragry, (1994). The mechanism of action of salicylanilides and their substituted phenol is through their action as uncoupler of oxidative phosphorylation
(Adams, 1995). The drug is more effective on adult than immature flukes (Richard et al., 1990). Both ivermectin and rafoxanide are commonly concurrently used for prophylaxis and treatment of nematodes, external parasites and Fasciola infestations in sheep and there is no available data concerning the untoward effects of their concurrent use. In support of the previous concept, the present study was designed to unearth the possible side effects of ivermectin, rafoxanide and their concurrent use as prophylactic drugs that would be portrayed as undesirable biochemical and hematological effects.

**MATERIAL AND METHODS**

1-Drugs:

A- (Ivermectin 1%) ® is produced by Kahira Pharmaceutical and Chemical Industries Company, Cairo, Egypt. The drug is available as injectable 1%, w/v sterile solution. The drug was administered subcutaneously as a single dose of 0.2 mg kg⁻¹ (Garg et al., 2007).

B- (Rafoxanide) ® is produced by the Egyptian Company for Chemical and Pharmaceuticals (ADWIA), the drug is available as injectable solution 7.5% each vials contains 100 ml. This drug was administered as single dose of 3mg kg⁻¹ (Adams, 1995).

2-Animal:

The present study was carried out on 20 apparently clinically healthy rams 40-50 kg. They were housed in open yard system and fed on dry ration and barseem ad-libitum. Rams were classified into 4 equal groups. The 1st group was left without treatment (control). The 2nd group was injected subcutaneously with ivermectin in a dose of 0.2 mg / kg⁻¹
(ivermectin treated group). The 3rd group was injected subcutaneously with rafoxanide in a dosage of 3 mg kg\(^{-1}\) (rafoxanide treated group). The 4th group was injected concurrently subcutaneously with both drugs 0.2 mg kg\(^{-1}\) (ivermectin) and 3 mg kg\(^{-1}\) (rafoxanide) (ivermectin and rafoxanide treated group).

3-Sampling:

Two blood samples were collected by jugular puncture from animals after one and two weeks post drugs administration. The 1st sample was collected in centrifuge tube without anticoagulant and used for separation of clear serum for biochemical analysis. The serum was separated by centrifugation at 300 r.p.m. for 15 minutes and kept frozen at – 20 °C till assayed. The 2nd sample was collected in a test tube containing EDTA as anticoagulant and used for hematological studies.

4-Analysis:

A- Biochemical analysis:-

The serum samples were used for determination of triglycerides (Bucolo and David, 1973), total cholesterol (Mellattini, 1978), HDL-c (Clark et al., 1983), LDL-c (Friedwald et al., 1972), Albumin (Gassbaro et al., 1972), blood urea nitrogen (Putton and Crouched, 1977), creatinine (Young et al., 1975) and serum AST and ALT levels (Reitman and Frankel, 1977).

B- Hematological analysis:-

The hematological parameters (Hb%, RBCs and WBCs counts and PCV %) were determined according to (Dacie and Lewis, 1994). Blood indices were calculated using these equations.
C- Statistical analysis:-

The results were reported as the mean ± S.E. Statistical significance was determined using analysis of variance according to (Snedcor and Cochran, 1982). Means were compared by Least Significance Difference (LSD) test at 0.5 significance level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Hematological findings:

Concerning the effect of s/c administration of ivermectin, rafoxanide and their concurrent use on hemogram (Table 1) and hematological indices (Table 2) of treated rams, it was indicated that, s/c injection of ivermectin (0.2 mg kg⁻¹) evoked non significant change in leukocytic count (after 1st and 2nd weeks) . On other side, there was a significant decrease in erythrocytic count PCV % and Hb% (after 1st and 2nd weeks) . The hematological indices revealed significant decrease in MCH, MCHC and MCV values (after 1st and 2nd weeks).It was cleared that, s/c administration of rafoxanide (3mg kg⁻¹) elicited significant decrease in leukocytic and erythrocytic counts, Hb concentration and PCV % (after 1st and 2nd weeks). The hematological indices emphasized a significant decrease in MCH , MCHC and MCV values (after 1st and 2nd weeks). Regarding the effect of concurrent s/c administration of both ivermectin (0.2 kg⁻¹) and rafoxanide (3mg kg⁻¹), it was found that there was significant decrease in leukocytic and erythrocytic counts, PCV % and Hb%( after 1st and 2nd weeks) as well as Hb concentration (after 1st
week) as compared with the control group. While, in comparison with ivermectin and rafoxanide treated groups, there was a significant decrease in RBCs count (after 1st and 2nd weeks) as compared with ivermectin and rafoxanide treated group as well as a significant decrease in Hb concentration (after 1st week) and PCV% (after 1st and 2nd weeks) as compared with ivermectin treated group. In contrast, there was non significant change in erythrocytic count, Hb% and PCV% (after 1st and 2nd weeks) as compared with rafoxanidetreated group. The hematological indices provoked significant decrease in MCH, MCHC, and MCV values (after 1st and 2nd weeks) while, in comparison with ivermectin treated group. In contrast, there was non significant change in MCH and MCHC% (after 1st and 2nd weeks) as compared with rafoxanide treated group.

Analysis of the hematological parameters can be beneficial in assessing animal health as the hemogram and blood indices reflect the statement of the animals. From these obtained results, it was evident that s/c administration of ivermectin, rafoxanide and their concurrent use decreases the erythrocytic count and Hb level of treated rams. Unfortunately, our findings can not provide us with a ready explanation for ivermectin induced anemia in treated rams. It is worthwhile to contemplate proposals that enlighten us with a proper explanation. The anemia may be due to alteration of haematopoiesis as a result of hepatic pathological lesions or might be due to reduction in bile salts in small intestine since bile acids are necessary for reduction of ferric ions to ferrous easily absorbed (Kaneko et al., 1997). In the glow of the previous notion one could attribute this ivermectin induced anemia to inhibition of haemopoiesis caused by hepatic pathological lesions. This suggestion confirmed by Roey et al., (1992), Zaied (1995), Wanis (1996) and Gad
(1998) and Zaied (2004). The authors stated that therapeutic dose of ivermectin induced various hepatic degenerative changes, cloudy swelling lymphocytic infiltration, coagulative necrosis and congestion of hepatic blood sinusoids. Moreover, this suggestion is further confirmed by Ali and Abu-Samra (1987) who reported that ivermectin treatment induced hepatitis in goats. On other side, these results are incompatible with that recorded by Asquith (1981) who stated that, i.m injection of ivermectin to adult horses at 0.2 or 0.3 mg kg\(^{-1}\) revealed no changes in hematological values. Also, these results were in disagreement with that reported by Berm and Bulman (1986) who recorded that the hematological values of cattle treated s/c with ivermectin at 0.2 mg kg\(^{-1}\) three times with 30 days intervals showed little or no effect at 1 and 7 day after each of three treatments. These data were also, incompatible with that detected by Mahzunlar (1989) and Nakai (1990) who found that dogs treated with ivermectin revealed no abnormalities for any variable measured at hematological examination. Also, these results were disagree with Roy et al., (1992) who showed that s/c treatment of 4 clinically healthy female goats with ivermectin at a dose of 0.2 mg kg\(^{-1}\) for 7 consecutive days revealed non significant changes in Hb levels, total erythrocytic count, total and differential leukocytic counts and blood clotting time. Moreover, these data were not coincided with Ragab (1994) who stated that, ivermectin injection 0.2 mg / kg\(^{-1}\) for 7 days in did not produce any untoward effects on Hb, RBCs, total and differential leukocytic counts. Additionally the results were in disagreement with those recorded by Wanis (1996) and who noted that male rats injected s/c with ivomec-f and ivermectin in dose of 0.2 and 0.3 mg / kg\(^{-1}\) respectively, reflected insignificant changes on erythrocytic and leukocytic counts, PCV\% and Hb concentration along the course of the experiment.
Some Hematological And Biochemical ...

Concern regarding Rafoxanide induced anemia, our findings were nearly fit with El-Sayed and El-Sayed (2000) who reported that s/c treatment of ewes with rafoxanide at 3 mg kg-1, induced reduction in Hb concentration (after 1\textsuperscript{st} week) and leukocytic and erythrocytic counts (after 2\textsuperscript{nd} week). Concerning anemia induced by concurrent use of both drugs, undoubtedly our findings obtained with other two treated groups provide us with a ready explanation and needless to say this could be attributed to augmented effect of both drugs.

**Biochemical findings:**

The results concerning tested serum parameters are summarized in Table (3). It was cleared that, s/c administration of ivermectin induced significant elevation in serum AST (after 2\textsuperscript{nd} week) and serum ALT (after 1\textsuperscript{st} and 2\textsuperscript{nd} weeks). Undoubtedly, our findings fit in with those reported by Roey et al., (1992) who found that s/c administration of ivermectin (0.2mg kg\textsuperscript{-1}) to 4 clinically healthy female goats for 7 consecutive days elicited an increase in serum AST and ALT levels. These results were also in accordance with Ragab (1994) who noted that female sheep injected with ivermectin (0.2 mg kg\textsuperscript{-1} for 7 consecutive days produced a significant increase in serum transaminases level. Moreover these results were also go parallel with Hassan et al., (1995), Zaied (1995) and Wanis (1996) and Zaied (2004). On other side, these data were in disagreement with that reported by Asquith and Kulwich (1981), Brem and Bulman (1986) and Uysal and Mahzunlar (1989). Also, s/c administration of ivermectin revealed non significant change in albumin, blood urea nitrogen, creatinine, cholesterol, triglycerides, HDL-c and LDL-c. Our results are in harmony with Asquith and Kulwich (1981), Brem and Bulman (1986), Nakai et al., (1990), Wanis (1996) and Zaied (2004).
Regarding the effect of rafoxanide treatment, it was emphasized that, s/c administration of rafoxanide (3mg kg\(^{-1}\)) provoked a significant increase in serum AST and ALT levels as compared with the control. Serum transaminases including AST and ALT are found in most tissues but in equal proportions. ALT occurs exclusively in the liver, but only in the cytoplasm of parenchymal cells, in contrast to AST which is equally distributed between the cytoplasm and mitochondria. *Doxy* (1971) stated that the level of these enzymes is increased following liver damage. Accordingly, could attribute an elevation of AST levels to the damage of hepatic cells by direct effect of the drug resulting escape of these enzymes to the plasmas. On other side, our findings were in disagreement with *El-Sayed and El-Sayed* (2000) who found that there was non significant change in ALT following s/c administration of rafoxanide to ewes. The present results were also in disagreement with *Bulent et al.*, (2006) who recorded that there was non significant change in AST following oral administration of rafoxanide to cattle. Rafoxanide treatment also revealed non significant change in albumin, blood urea nitrogen, creatinine and HDL-c. These results were nearly correlate with *Swan and Schroder* (1981) and *Shroder* (1982) who stated that rafoxanide is save in sheep and cattle when administered at the recommended therapeutic dose. Also, these findings were coincided with *Adams* (1995) who concluded that the therapeutic dose of rafoxanide is save in cattle and sheep of all ages without any hazards. Moreover, these results were nearly similar to those obtained by *El-Sayed and El-Sayed* (2000). The s/c administration of rafoxanide to rams also elicited a significant increase cholesterol and LDL-c levels, meanwhile significant
decrease in triglycerides level (after 1\textsuperscript{st} and 2\textsuperscript{nd} weeks). Our data confirmed by \textit{El-Sayed and El-Sayed (2000)} who concluded that s/c administration of rafoxanide to ewes produced an elevation in total cholesterol (after 2\textsuperscript{nd} week) and LDL-c (after 1\textsuperscript{st} and 2\textsuperscript{nd} week) levels. The concurrent injection of both ivermectin (0.2mg kg\textsuperscript{-1}) and Rafoxanide (3mg kg\textsuperscript{-1}) provoked a significant increase in serum AST and ALT levels as compared with control, ivermectin and rafoxanide treated groups. This elevation might conceivable to be due to the effect of both drugs. This suggestion was confirmed by obtained data with ivermectin and rafoxanide treated groups in this study. Also, rams treated s/c with both ivermectin (0.2mg kg\textsuperscript{-1}) and rafoxanide (3mg kg\textsuperscript{-1}) concurrently showed non significant changes in albumin, blood urea nitrogen, creatinine and HDL-c levels (after 1\textsuperscript{st} and 2\textsuperscript{nd} weeks) as compared with control, ivermectin and rafoxanide treated groups. Undoubtedly, our findings obtained with ivermectin and rafoxanide treated groups provide us with a ready explanation and needless to say these results coincided with that recorded by \textit{Asquill and Kulwish (1981), Berm and Bulman (1986), Naka et al., (1990), Poul et al., (1993), Adams (1995) and El-Sayed and El-Sayed (2000)} who concluded that both drugs are safe drugs. Moreover, rams treated s/c with ivermectin and rafoxanide concurrently indicated a significant decrease in serum triglycerides level as compared control and ivermectin (after 1\textsuperscript{st} and 2\textsuperscript{nd} weeks) as well as rafoxanide (after 2\textsuperscript{nd} week) treated groups. This effect could be attributed to rafoxanide as obtained in this study and confirmed by \textit{El-Sayed and El-Sayed (2000)}. 
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"بعض التغيرات الدموية والبيوكيميائيه الناتجة عن الاستخدام المتزامن للأيفرميكتين والرافيوكسانيد في الكباش

د./ أشرف أحمد الغنيمى و د./ أسامة بهجت الجزار "

قسم الأدوية والكيمياء الحيوية - كلية الطب البيطري - قنا / جامعة جنوب الوادي

يستخدم كلا من الأيفرميكتين و الرافيوكسانيد على نطاق واسع لعلاج الديدان الداخلية والفصيل في الأغنام. أجريت هذه الدراسة على عدد 20 من الكباش البالغة السليمة ظاهريا وذلك لمعرفة بعض التغيرات الدموية والكيميائيه الناتجة عن استخدام كلا من هذين العقراين على حده وكذلك استخدامهما لمتبنايم. قسمت هذه الكباش إلى أربعة مجموعات متساوية قوام كل مجموعة خمسة

Afrad. The first group consisted of one group of sheep treated with a single dose of 0.2 ml/kg of ivermectin, while the second group consisted of the same number of sheep treated with a single dose of ivermectin at 3 mg/kg. The third group consisted of the same number of sheep with no treatment. The fourth and fifth groups consisted of a group of sheep treated with a single dose of ivermectin (0.2 ml/kg) and a group of sheep treated with ivermectin (3 mg/kg), respectively. The sixth group consisted of the same number of sheep with no treatment.

In addition to the above treatments, the following tests were performed:

1. The total number of red blood cells (RBC), hemoglobin concentration, and platelet count were determined.
2. The level of enzymes in the serum and the level of creatine kinase and aspartate transaminase were determined.
3. The level of lipids, cholesterol, and triglycerides in the serum was determined.
4. The level of urea and creatinine in the serum was determined.
5. The level of blood gases in the serum was determined.
6. The level of electrolytes in the serum was determined.
7. The level of protein in the serum was determined.
8. The level of immunoglobulins in the serum was determined.
9. The level of other biochemical parameters in the serum was determined.

The results showed that there were no significant differences in the biochemical parameters between the treatment groups and the control group. However, there were significant differences in the level of urea and creatinine in the serum between the treatment groups and the control group. The level of urea and creatinine in the serum was significantly higher in the treatment groups than in the control group.

In conclusion, the results of this study indicate that ivermectin is safe and effective in the treatment of hematological and biochemical parameters in sheep.
مستويات الزلال، اليوريا، الكربانتين، الدهون عالية الكثافة وعدد كريات الدم الحمراء. على الجانب الآخر كانت هناك زيادة معنوية في مستويات أنزيمات الألدنين والأسيرتيت ترايسفيير، الكولستيرون والدهون منخفضة الكثافة بمصل الدم مقارنة بكل من المجموعة المعالجة بالأيفرميكتين وكذلك المجموعة المعالجة بالرابانوكساديد.

وبالمقارنة بالمجموعة المعالجة بالأيفرميكتين كان هناك نقص معنوي في نسبة اليموجلوبين، حجم الكريات المرصوصة، متوسط حجم كريات الدم، متوسط هيموجلوبين الخلية ومتوسط تركيز هيموجلوبين الخلية. وفي المقابل كانت هناك زيادة معنوية في مستوى الكولستيرون بمصل الدم. ومقابل هذه المجموعة مع المجموعة المعالجة بالراووكساديد لم يكن هناك أي تغيرات معنوية في مستويات الكولستيرون والدهون الثلاثية بمصل الدم، عدد كريات الدم الحمراء، نسبة اليموجلوبين، حجم الكريات المرصوصة، متوسط هيموجلوبين الخلية ومتوسط تركيز هيموجلوبين الخلية.

وقد خلصت هذه الدراسة إلى أن التغيرات الدموية والبيوكيميائية الناشئة عن الحقن المتزامن بكلا من عقاري الأيفرميكتين والراوكساديد لا تختلف عن تلك التي نتجت عن حقن كلا من العقرين على حدة، و بناء عليه لا توجد خطورة معاقة من ذلك الاستخدام.