# A COMPARATIVE STUDY ON THE ANTISCHISTOSOMAL ACTIVITY OF PRAZIQUANTEL AND THREE DERIVATIVES OF ITS HYDROLYSIS PRODUCT AGAINST *S. MANSONI*

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تم اتباع طريقة جديدة لمعرفة العلاقة بين التركيب الكيميائي والفاعلية لدواء البرازيكوانتل **(I)** وذلك بفتح حلقة اللاكتام واشتقاق المركب الناتج وهو: N-(1,2,3,4-تتراهيدرو-1-أيزوكينولينايل-ميثيل)-N-سيكلوهكسيل-كربونيل جلايسين**(II)**. وبهذه الطريقة تم تحضير ستة عشر مركباً من ثلاث طوائف كيميائية مختلفة هي 2-ألكيل أيزوكينولينايل، و2-أسيتيل أيزوكينولينايل، و 2-سلفانيلاميد وأيزوكينولينايل. من هذه المركبات تم اختيار المركب N-(1,2,3,4-تتراهيدرو-2-أسيتيل -1-أيزوكينولينايل-ميثيل)-N-سيكلوهكسيل-كربونيل جلايسين**(III)**، والمركب N-(1,2,3,4-تتراهيدرو-2-أيثيل -1-أيزوكينولينايل ميثيل)-N-سيكلوهكسيل-كربونيل جلايسين**(IV)** والمركب N-(1,2,3,4-تتراهيدرو-2-سلفانيلاميدو-1-أيزوكينولينايل ميثيل)-N-سيكلوهكسيل-كربونيل جلايسين**(V)** كمركبات ممثلة لكل سلسلة من المركبات وتم اختبار فاعليتها ضد داء البلهارسيا (الشستوسوما مانسوني) في الفئران البيضاء. وقد تم إعطاء هذه المركبات للحيوانات فموياً بجرعة 40مغ/كغ من وزن الجسم( $10^{-4}$ x 0.08 – 1.28عياري). واستخدمت الجرعة المنخفضة لكشف الفروقات في الفاعلية والسمية. كما تم استخدام بارامترات المؤشرات المختلفة وذلك لتقييم فاعلية هذه المركبات. إذ أن مجموعات الحيوانات المعالجة بالبرازيكوانتل **I**، وبالمركب **III** وبالمجموعة الشاهدة المصابة بالمرض قد أظهرت أقصى حد لإفراز البيوض في البراز في الأسبوع العاشر بعد الإصابة بينما أظهرت المجموعة المعالجة بالمركب **V** أقل عدد، وأما المجموعة المعالجة بالمركب **IV** فقد أوقفت إفراز البيوض تماماً في الأسبوع 9.5 بعد الإصابة. وقد أظهرت كل المجموعات المعالجة تعداداً للبيوض في الكبد والأمعاء أقل من الموجود في المجموعة الشاهدة المصابة. وقد دلت نسبة الانخفاض في عدد الديدان في المجموعات المعالجة بالبرازيكوانتل = 16.6% ، وبالمركب **III**= 42.8% ، وبالمركب **IV**= 100% ، وبالمركب **V** = **88%** وذلك بالمقارنة مع المجموعة الشاهدة المصابة. إن هذه المركبات كانت أكثر فاعلية في خفض الديدان من البرازيكوانتل. وأظهرت كل المركبات تأثيراً ضد داء الشستوسوما (البلهارسيا) على الديدان الإناث أكثر منه على الديدان الذكور. وقد كانت الأضرار الكبدية المتمثلة في الورم الحبيبي الخلوي ، وزيادة سماكة القناة البابية ، وانسداد ورمي حبيبي وعائي، وبؤرات الكريات البيضاء، والالتهاب حول الوريد، وتردُف الوريد المركزي، وتخضب خلايا كوبفر المتكاثرة والمتضخمة ظاهرة بوضوح في المجموعة الشاهدة المصابة وفي مجموعة المركب **III**. وقد كان الانخفاض في متوسط عدد الأورام الحبيبية الكبدية وحجمها مترابطاً مع النتائج الإكلينيكية الباثولوجية. وبناءً على المؤشرات السابقة فإن المركب **IV** له فاعلية أعلى ضد داء الشستوسوما (البلهاسيا) بالمقارنة مع دواء البرازيكوانتل.

A new approach to the structure activity relationship of praziquantel (I) has been adopted in this work, through the opening of the lactam ring and derivatization of the resultant product N-(1,2,3,4-tetrahydro-1-isoquinolinyl-methyl)-N-cyclohexyl-carbonyl glycine (II). In this approach sixteen compounds of three

different classes of compounds (2-alkyl isoquinolinyl, 2-acetyl isoquinolinyl and 2-sulphanilamide isoquinolinyl) have been prepared . From these compounds; N-(1,2,3,4-tetrahydro-2-acetyl-1-isoquinolinyl-methyl)-N-cyclohexyl-carbonyl glycine (III), N-(1,2,3,4-tetrahydro-2-ethyl-1-isoquinolinylmethyl)-N-cyclohexyl carbonyl glycine (IV) and N-(1,2,3,4,-tetrahydro-2-sulphanilamido-1-isoquinolinylmethyl)-N-cyclohexyl-carbonyl glycine (V) were selected as representatives for each series of compounds and tested for antischistosomal activity against *S. mansoni* in Albino mice. These compounds were given orally to the animals in a single oral dose of 40 mg/kg body weight ($0.08 – 1.28 \times 10^{-4}$ mole). The low dose used to detect differences in efficacy and toxicity. Different indicator parameters were used to assess the activity of these compounds. The groups of animals treated with praziquantel (I), compound III and the control infected group showed a maximal faecal egg shedding in week 10 post infection while the group treated with compound V showed a minima and that treated with compound IV stopped faecal egg shedding completely on week 9.5 post infection. All treated groups showed lower liver and intestine egg count than that detected in the infected control. The percentage of worms reduction in the treated groups (PZQ = 16.6%, III = 42.8%, IV = 100% and V= 88% compared to infected control group) indicated that these compounds were more effective on worms reduction than praziquantel. All compounds showed stronger antischistosomal effect on female worms than on males. The hepatic lesions, comprising mainly of cellular granuloma, portal tract thickening, vascular granulomatous occlusion, leucocytic foci, periphelebitis, central vein cuffing and pigmentation of the proliferative and swollen kupffer cells were highly marked in infected untreated control and compound III groups. Reduction of the mean number of hepatic granulomas and their size were strongly correlated with the clinicopathological findings. Based on the above mentioned parameters compound IV was found to possess higher antischistosomal activity compared to praziquantel.

***Key words:*** Anthelmintics, praziquantel, derivatives, Structure-activity relationship, clinico- and histopathology.

## Introduction

*Schistosoma mansoni* is widely distributed throughout tropical and subtropical African countries (1). Many drugs have been used for the treatment of schistosomiasis, a disease which characterized by dysenteric symptoms and in severe cases by fibrosis of the liver and splenomegaly ascitis (2-4). Praziquantel (2-cyclohexyl carbonyl-1,3,4,6,7,11b-hexa hydro-2H-pyrazino-(2,1-a) isoquinoline-4-one, Fig. 1) has been used as the drug of choice for the treatment of this disease. The drug is reported to be safe and effective against all schistosomes and cestodes species (5-8), with a high cure rate. However, existence of resistance to praziquantel has recently been reported in a laboratory produced strain of *S. mansoni*, which decrease its efficacy as anthelmintic drug (9-12).

Many studies and reports on the structure-activity relationship of praziquantel emphasized the importance of the 4-oxo-group, six-membered cyclic-acyl group in position-2 and the ring system without any modification (13).

However, in the present work a new era of structure-activity relationship of praziquantel have been adopted, and the essentiality of the pyrazinoxy isoquinoline ring system for anthelminitc activity was tested, through the opening of the lactam ring and formation of various substituents at the functional groups of the resulting compound (OLF-PZQ II), in hope to produce an effective anthelmintic drug to act as an alternative.

## Materials and methods

*Compounds tested*

I praziquantel

II N –(1,2,3,4 – tetrahydro –1- isoquinolylmethyl) –N- cyclohexyl carbonyl glycine.

III N –(1,2,3,4 – tetrahydro –2-acetyl –1-isoquinolylmethyl) –N- cyclohexyl carbonyl glycine.

IV N –(1,2,3,4 – tetrahydro –2-ethyl –1- isoquinolylmethyl) –N- cyclohexyl carbonyl glycine.

V N –(1,2,3,4 – tetrahydro –2-(4-aminobenzene sulphonyl)–1-isoquinolylmethyl)–N-cyclohexyl carbonyl glycine.

*Animals and experimental design:*

Eighty four adult Albino mice, aged 8-12 weeks, weighing 20-25 gm, were used in this study. Animals were divided into two groups. One group of 12 mice was kept as a healthy untreated control group. The other group [72 mice] was infected with cercariae of *S. mansoni*. All the animals were kept at the same laboratory conditions and allowed food and tap water freely.

*Parasitological techniques:*

*Schistosoma mansoni* cercariae were obtained by infecting *Biomphalaria pfeifferi* snails collected from El Kryab village (North of Khartoum) with miracidia obtained from Schistosome eggs isolated from the faeces of patients infected with *S. mansoni* at Abu-Oshar, Gezira province, Sudan.

The mice in Group 2 [72] were infected by immersing them , separately, half to abdomen, in 15 ml tap water containing 100 cercariae for 45 minutes for each mouse . Faecal egg counts were made according to Bethony *et al* (14) and tissue counts by the KOH digestion method described by Cheever (15). Worms were recovered and counted by the perfusion technique described by Bogh *et al* (16).

*Treatment and dose:*

All the tested compounds were prepared in a concentration of 40 mg per 10 ml of 15% aqueous propylene glycol. Seven weeks post infection the infected mice were divided into six groups. Each member of the same group received an oral single dose of 0.25 ml of the prepared compound solution (equivalent to 40 mg/kg body weight or 1.282, 1.212, 1.075, 1.117 and .8230 x $10^{-4}$ moles / kg body weight of I, II, III, IV and V respectively). Treatment was conducted when eggs first appear in faeces.

*Biochemical analysis:*

Ten weeks post infection, each mouse was anesthetized, opened and blood was directly withdrew from the heart – about one ml. Blood samples were centrifuged for serum separation which was then kept at freezing point till used. Serum Glutamic-oxaloacetic transaminase (GOT) and Glutamic-pyruvic transaminase(GPT) estimations were performed using a commercial test kit (Plasmatic Lab-products, U.K.).

*Histopathological techniques:*

Specimens of livers, intestines of the different groups were fixed in 10% formal saline, processed, sectioned and stained with haematoxylin and eosin (H & E).

*Statistical analysis:*

All statistical analysis were carried out using SPSS statistical package for windows, version 7.5. The statistical analysis was carried out at $p > 0.05$ using student t – test.

## Results

Faecal egg count was conducted to assess the effects of the test compounds (Fig. 1) on oviposition. The follow up was made for 10 weeks post infection. The groups of animals treated with compounds praziquantel (I) and (III) as well as the infected control group showed their maximum egg shedding on week 10 post infection when the experiment was terminated (Table 1, Fig. 2). These results showed that the groups treated with compound I and III exhibited a reduction of 76% and 58% respectively compared to the untreated infected control. Unlike other groups, the group treated with compound (V) showed it's minimum egg count on week 10 post infection. The group treated with compound (IV) was exceptionally interesting in that egg shedding in this group stopped completely on week 9.5 post infection (2.5 weeks post treatment) and the faeces continued to be free of eggs till the end of the experiment.

The number of worms recovered in all treated groups of animals was lower than that of the infected control (Table 2). The reduction in worms, compared to infected control group, in groups treated with compounds praziquantel (I), III, IV and V was 16.6%, 42.8%, 100% and 88%, respectively. These results clearly indicate that all the compounds tested possess greater antischistosomal effect than praziquantel (I). There were significant differences ($p < 0.05$) in the reduction of the total number of worms, between the infected control group and the groups treated with compounds III, IV and V. On the other hand, significant differences ($p < 0.05$) were noted in the reduction of female worms between the infected control group and all the other groups particularly group, IV and III.

All treated groups showed lower liver and intestine egg count than that of the infected control. The lowest liver egg count was found in the group treated with compound IV followed by groups treated with compounds I, V and III and the infected control group, respectively. Again the lowest intestine egg count was observed in the group treated with compound V followed by the groups treated with compound IV which showed the lowest liver and intestine egg counts. However, the reduction in the liver and intestine egg count of all treated groups, compared to that of the infected control group were statistically non significant .

With exception of the group treated with compound III, all other treated groups showed non significant differences in the values of GPT values from that of the healthy control. Groups treated with compounds PZQ, I, showed non significant differences in the GOT values from that of the healthy control (Table 3).

The lesions and granulomas of the liver were typical of schistosomiasis. The number of granulomas varied between 4 and 41. The size of the granulomas ranged from 114 to 228 µm .

Intestine showed heavy infiltration of inflammatory cells in the mucosa replacing the mucosal gland, destruction and erosion of mucosa and intraglandular infiltration of inflammatory cells. Mucosal glands are found near the muscularis mucosa which showed hyperplasia and hypertrophy and some parts were infiltered with inflammatory cells. Mucosal glands showed mucous degeneration .

## Discussion

The tested compounds, have been chosen as representatives of sixteen compounds derived from the alkaline hydrolysis product of praziquantel (the open lactam form of praziquantel, OLF-PZQ II, Figure 1) which were synthesized and characterized in our laboratories. These derivatives are believed to prevent the biocyclization of the open lactam form to the parent praziquatel. Hence any effect produced by these compounds could only be due to the new introduced compounds. They differ from that of praziquantel in the lactam ring system.

Higher dose of praziquantel is usually used to treat mice infected with *Schistosoma mansoni* because mice are reported to rapidly metabolize praziquantel (12,17). However, in this experiment low dose of praziquantel and its derivatives has been used from comparative reasons to detect differences which might appear in toxicity or efficacy in the groups treated with the different compounds compared to that treated with praziquantel. However in terms of moles the dose given for praziquantel ($1.282 \times 10^{-4}$ mol) was the highest one among the other tested compounds, ($1.212$, $1.075$, $1.117$ and $0.8 \times 10^{-4}$ mol of compounds II, III, IV and V respectively).

Based on the degree of worm reduction, this study showed that all the compounds investigated possess higher antischistosomal activity than praziquantel. The degree of worm reduction (100%) by the group treated with the dose used with compound (IV) illustrated that this compound possesses the highest efficacy. This could be attributed to an intrinsic activity of this compound or to the pharmacokinetic properties of the compound, in the animal tested, compared to that of praziquantel. This high activity was also apparent from the faecal egg count. Eggs in faeces disappeared completely on week 9.5 post infection in the group treated with compound IV. Compound (V) showed a somewhat high degree of worm reduction (88%). This could explain the gradual and continuous decline in faecal egg counts recorded for this group of animals over the three weeks post infection.

All treated groups provided lower liver and intestine egg count than that observed in the infected control group. The group treated with compound IV showed the least tissue egg count. For other treated groups the order of reduction in liver egg count was not similar to that in intestine. A compound that gave low liver egg count may not necessarily give low intestine egg count, and a compound that show a high intestine egg load may not show a high faecal egg shedding. The difference in egg shedding and oviposition obtained in this study for the tested compounds could be attributed to the variable effect of these compounds on oviposition or on faecal egg shedding (18-21).

**Table 1.** Egg count per gram of feces of Albino mice infected with *S. mansion* and treated.

| Group | N | Weeks post infection | | | | |
|---|---|---|---|---|---|---|
| | | 7 | 8 | 9 | 9.5 | 10 |
| | | Weeks post treatment | | | | |
| | | 0 | 1 | 2 | 2.5 | 3 |
| Infected untreated control | 12 | 379 | 424 | 573 | **800** | **2624** |
| Comp. I treated | 12 | 379 | 400 | 460 | **540** | **615** |
| Comp. II treated | 12 | 379 | 430 | 485 | **600** | **685** |
| Comp. III treated | 12 | 379 | 413 | 491 | **614** | **658** |
| Comp. IV treated | 12 | 379 | 392 | 179 | **00** | **00** |
| Comp. V treated | 12 | 379 | 409 | 396 | **267** | **151** |

**Table 2.** Antischistosomal effect of praziquantel and related derivatives on *S. mansoni* in Albino mice.

| Group | n | Mean number of worms per mouse (± SD) | % reduction of worms | Sex ratio M:F |
|---|---|---|---|---|
| Infected untreated control | 12 | 42 ± 4 | --- | 11:10 |
| Compound I treated | 12 | 35 ± 5.6 | 16.6 NS | 5:2 |
| Compound II treated | 12 | 30 ± 5 | 28.6 * | 7 : 3 ** |
| Compound III treated | 12 | 24 ± 4 | 42.8 * | 3:1 ** |
| Compound IV treated | 12 | Zero | 100 * | -- ** |
| Compound V treated | 12 | 5 ± 2 | 88 * | 3:2 ** |

NS not significant
* significant at p = 0.05
** significant at p = 0.05 only for female worms

**Table 3.** Activities of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and total protein in plasma of Albino mice

| Group | n | GOT u/l mean ± SD | GPT u/l Mean ± SD |
|---|---|---|---|
| Infected untreated control | 12 | 63 ± 13.75 * | 15 ± 5.2 NS |
| Healthy control | 12 | 17 ± 4.58 | 6.6 ± 2.09 |
| Comp. I treated | 12 | 35.6 ± 12.98 NS | 7.05 ± 3.71 NS |
| Comp. II treated | 12 | 34.2 ± 7.22 * | 12.3 ± 7.6 NS |
| Comp. III treated | 12 | 9.5 ± 2.29 * | NA |
| Comp. IV treated | 12 | 45.3 ± 2.5 * | 8.1 ± 1.5 NS |
| Comp. V treated | 12 | 54.62 ± 21.64 * | 16.15 ± 8.48 * |

NA = not available
* significant at p = 0.05
NS not significant

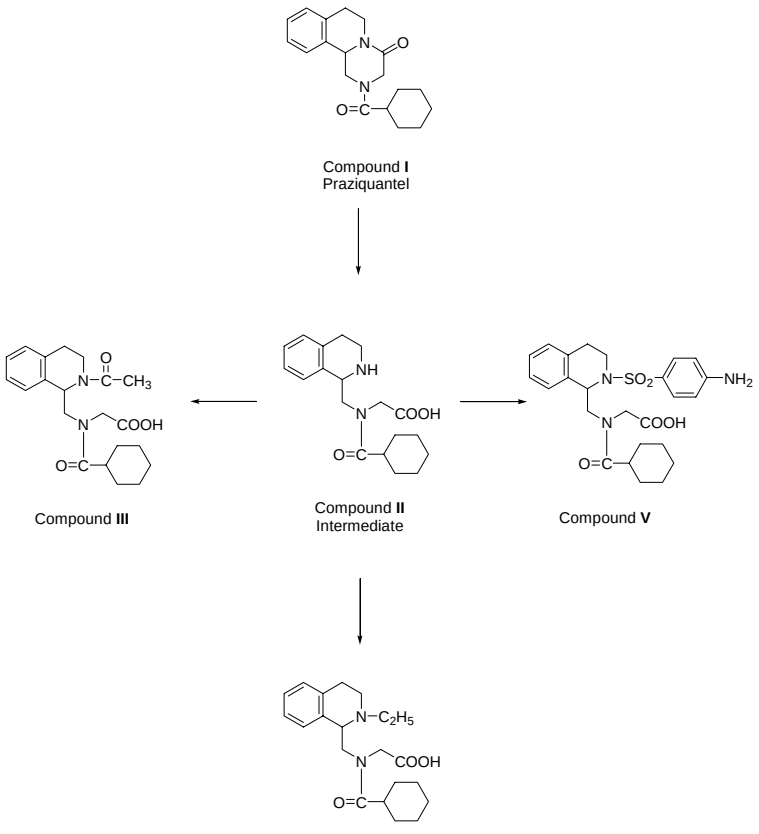


**Figure 1:** Chemical structures of the tested compounds.

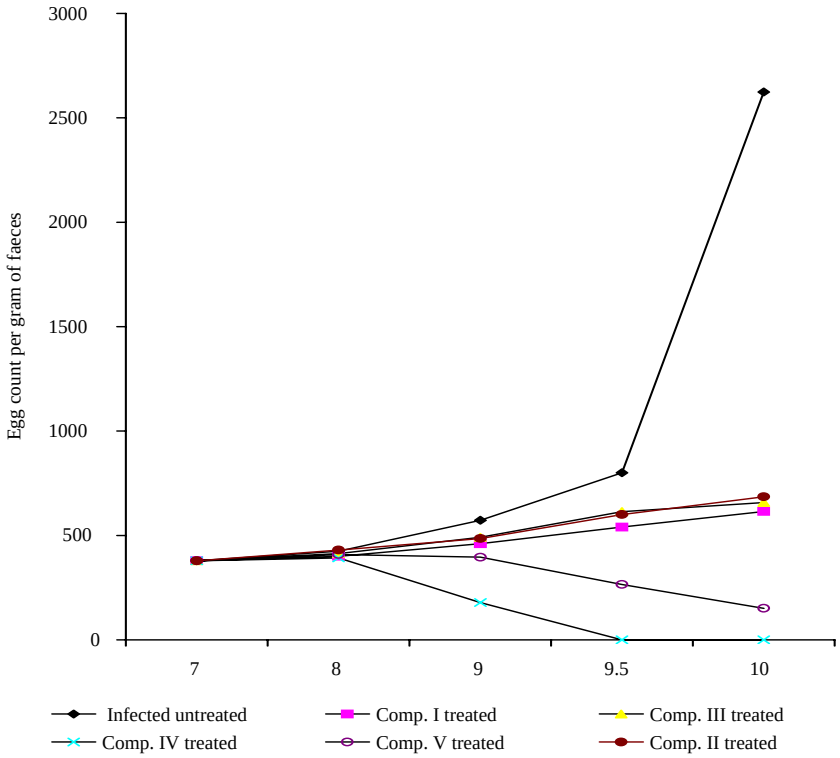


**Figure 2:** Profile of *Schistosoma mansoni* egg shedding in mice.

Activity of both Glutamic-oxaloacetic transaminase (GOT) and Glutamic-pyruvic transaminase (GPT) enzymes were higher in the infected untreated control and animals treated with compound V as compared to mice treated with compound I, IV and the uninfected control. It is known that the changes in serum concentration of these enzymes is used to assess the liver performance and evaluate the degree of destruction.

It is evident from tissue egg deposition that a mean of 5200 and 4074 eggs were deposited per grams of liver of mice in the infected untreated group and group V as compared to 3690 and 2745 recorded for group I and IV .The higher liver egg count in the infected untreated control and animals treated with compound V may explain the high values of these enzymes in these groups.

Deposition of eggs in the liver of the group treated with praziquantel (I) was higher than that of compound IV, though the group treated with compound IV showed a lower GOT and GPT levels than that of praziquantel group. This could be due to the suppressed egg viability induced by praziquantel (18,19). Since only viable eggs were reported to provoke granulomations reactions at the expense of hepatic parenchyma (22).

It is evident from the present study that the main typical schistosomiasis lesions caused by eggs were found in all groups, though they varied in degree and intensity. The presence of these lesions was obviously due to the presence of eggs before administration of these compounds. The egg laying usually starts by some worms at the 6th week post infection and by the 8th week the whole worm

population has begun egg deposition.

The results revealed that the mean number of hepatic granulomas in compound IV treated group was reduced by 60% as compared to infected untreated control during the 2½ weeks of oviposition, indicating and confirming the high potency of this compound which had eliminated the whole worm population. On the other hand, the average size of the granulomas (168 µm) was less but comparable to control infected untreated (174 µm) indicating that this compound – IV – has very little effect on egg viability.

A reduction of 66% in number of hepatic granulomas was observed for compound V treated group as compared to infected untreated control which could explain also the high efficacy of this compound. It should be emphasized that both compounds IV and V groups has almost similar upper limit of the granulomas range and that average number of the intestinal granulomas for both groups was similar.

Conversely, the lowest in average number of granulomas reduction 32% was calculated for compound III group indicating the inferiority of this compound as compared to compound IV and V groups.

The hepatic lesions, comprising mainly of cellular granulomas, portal tract thickening, vascular granulomatous occlusion, leucocytic foci, periphelebitis, central vein cuffing and pigmentation of the proliferative and swollen kupffer cells were highly marked in infected untreated control and compound III treated groups. However, these lesions were moderate in compounds IV and V groups, for example abundant and multiple leucocytic foci, heavy infiltration of inflammatory cells between the hepatic cords and cells, sinusoidal dilatation and high frequency of vascular granulomatous occlusion were only seen in compound III and infected untreated control groups.

Similarly, the intestinal lesions varied in intensity and severity between the different groups. Compound IV treated group showed moderate lesions characterized by mucosal and submucosal cellular granulomas, mild destruction of intestinal mucosan and interglandular infiltration of inflammatory cells.

Intestinal lesions in Compound V treated group were relatively severer than in compound IV treated group and were manifested by erosion and destruction of mucous membrane, heavy mucous degeneration of mucosal glands and inter- and intraglandular infiltration of inflammatory cells.

Compound III treated group showed the severest lesions which could be compared to the infected untreated control. The errosion and destruction was very marked, mucous degeneration was very extensive, heavy inter and intraglandular infiltration of inflammatory cells, cystic mucosal glands and replacement of glands by inflammatory cells.

The histopathological results indicate that the clinico pathological changes could be related closely to faecal egg counts, tissue egg counts and worm recovery, which all confirmed the high efficacy of compound IV seconded by V.

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