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PRELUDE TO THE ADVERSE EFFECTS OF LEVAMISOLE AND NICLOSAMIDE USED CONCEMTTENTLY

(With 1 Table 6 Figures)

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دراسة الاثار الجانبية لاستخدامات الليفاميزول والنيكلوزاميد

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تم دراسة تأثير عقارى الليفاميزول والنيكلوزاميد على القلب والكبد والكلى والطحال فى الجرزان البيضاء حيث تم تقسيم الحيوانات الى اربعة مجموعات حقنت المجموعة الاولى تحت الجلد بالماء المقطر وحقنت المجموعة الثانية بعقار الليفاميزول تحت الجلد والمجموعة الثالثة تم اعطائها النيكلوزاميد عن طريق الفم والمجموعة الرابعة تم حقنها تحت الجلد وبالليفاميزو واعطائها النيكلوزاميد عن طريق الفم.

وقد اوضحت النتائج ان الليفاميزول يؤدى الى زيادة معنوية فى الالينين امينوترانس فيرايز والاسبرتات امينوترانس فيريز واليوريا بينما النيكلوزاميد قد ادى الى زيادة معنوية فى الترانس امينيز والبلوروبين واليوريا

وبالاشارة الى التأثيرات الهستوباثولوجية وجد ان اعطاء الليفاميزول يسبب استحالات بعضلة القلب والتهاب كلوى تسممى واستحالات فى الكبد وتنكرز فى الخلايا الليمفاوية بالطحال بينما يؤدى اعطاء النيكلوزاميد الى استحالات بعضلة القلب ولا يؤدى الى تغيرات واضحة فى كل من الكلى والكبد والطحال.

عند اعطاء العقارين معا اظهرت الدراسة وجود بؤر تنكرزية بالقلب بالاضافة الى استحالات بعضلة القلب والتهاب كلوى تسممى وبؤر تنكرزية بالكبد وبالخلايا الليمفاوية بالطحال.

هذا وقد استخلصت الدراسة ان الليفاميزول له اثار تسممية على الكبد والكلى والقلب خاصة عند اعطائه مع النيكلوزاميد.

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SUMMARY

The effect of anthelmintic agents, levamisole and niclosamide on the liver, kidney, heart and spleen were studied in albino rats. Thirty two adult male albino rats weighing 200 ± 15 gm. each, were used in this study. The animals were divided into four equal groups. Rats of gp. I were S.C. injected with distilled water and used as a control. Rats of gp. II were injected S.C. with levamisole Hcl 36 mg/Kg. b.wt. . That of gp. III were administrated orally with niclosamide 450 mg/Kg. b.wt. . The rats of gp. IV were injected S.C. with levamisole 36 mg/Kg. b.wt. concmitently niclosamide 450 mg/Kg. b.wt. was given orally. The results revealed that, levamisole induced a significant increase in the activities of alanine aminotransferase, aspartate aminotransferase and urea, while alkaline phosphatase, bilirubin and creatine did not show any significant changes. Niclosamide produces a significant increase in the activities of serum transaminase only. The combination of levamisole and niclosamide induced a significant increase in the alkaline phosphatase, AST, ALT, bilirubin, urea and creatinine. Regarding the histopathological changes, the administration of levamisole to rats resulted in myocardial degeneration, toxic nephrosis, mild hepatic degenerative changes and mild lymphocytic necrosis in the spleen. The niclosamide produces more extensive myocardial degeneration than levamisole with no pronounced alteration in the liver, kidney, and spleen. The combination of both drugs, produce focal myocardial necrosis in addition to the myocardial degeneration, toxic nephrosis, focal hepatic necrosis and lymphocytic necrosis in the spleen. It could be concluded that, levamisole Hcl is cardiotoxic, nephrotoxic drug specially when given with niclosamide.

INTRODUCTION

Levamisole, belongs to the chemical group of imidazothiazoles. It is the L. isomers of the broad spectrum anthelmintic tetramisole *(KATZUNG, 1987)*. It is a highly effective broad spectrum nematocide specifically acting on lung worms *(ALEXANDER, 1985)*. *MARRINER (1986)* stated that the therapeutic index of levamisole was low, especially in dogs, cats and horses. Niclosamide is a halogenated salicylanilide derivative. It is a very effective agent for treating most infections with cestodes in animals and man *(KEELING, 1968)*. Tetramisole in combination with niclosamide could be used as an effective treatment against infestation with different species of round, tape and lung worms *(DELGADO and PRIETO, 1971)*.

Levamisole has been shown an immunostimulant in both man and expermintal animals *(RENAUX, 1980)*. *AKELA* et al. *(1989)* found that levamisole when administrated at a dose and time schedule higher or larger than the recomended anthelmintic schedule, it supresses the immuno response.

DUKES (1980) stated that tetramisole should not be given in severe hepatic diseases or in combination with any hepatotoxic anthelmintic. On the other hand, *JONES* et al. *(1981)* reported that there were no specific contraindications in the administration of tetramisole with other anthelmintics. So, the aim of the present work was to detect the effects of administration of levamisole and niclosamide on the hepatic and renal functions.

MATERIAL and METHODS

I- Drugs:

1) Levamisole^{R} (levamisole Hcl injection 7.5%) was obtained from CID/pharmachim Bulgaria.

2) Yomesan^{R} (Niclosamide tablets 0.5 mg) suspended in distilled water (Bayer) Germany. Doses were calculated according to *PAGET and BARNES (1964)*.

II- Laboratory animals:

Thirty two adult male albino rats weighing 200 ± 15 gm. each, were used in the study.

III- Experiment:

The animals were divided into four equal of eight. The first group (gpI) was subcutaneously (S.C.) groups injected with distilled water in the neck region and was served as a control. Rats of the second group (gpII) were injected S.C.

with a single therapeutic dose of levamisole Hcl (36mg./Kg.B.WT.) The third group (gpIII) was administrated orally with a therapeutic dose of niclosamide (450mg./Kg.B.WT.) using a metalic stomach tube. The fourth group (gpIV) was injected with the therapeutic dose of levamisole Hcl concomitently the therapeutic dose of niclosamide was given orally.

Blood samples were collected by orbital sinus puncture, 24 hours after treatment by heparinized micropipettes. Samples were centrifuged at 3000 rpm for 15 minutes and sera were collected for enzymes assay. Serum samples were analysed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities *(REITMAN and FRANKEL, 1957)*; alkaline phosphatase (ALP) activity *(KING and WOOTON, 1959)*; urea *(WOOTON, 1964)*; creatinine *(JACKSON, 1975)* and bilirubin *(JENDERASSIK and GRAF, 1938)*.

For histopathologial studies, specimens from the heart, kidney, liver and spleen of both control and treated rats were fixed in 10% neutral buffered formalin for 48 hours. The fixed speimens were dehydrated in ascending grades of ethyl alcohol, cleared in zylene, embedded in parafin wax, sectioned at 4-6 microns thickness and stained with hematoxylin and eosin *(HARRIS, 1898)*.

The statistical analysis and significance of difference were carried out for the obtained data according to the methods of *SNEDECOR and COCHRAN (1967)*.

RESULTS

Injection of rats with a therapeutic dose of levamisole, resulted in a significant increase in the activities of AST, ALT and urea, while alkaline phosphatase, bilirubin and creatinine did not show any significant variation as compared to control animals (Table 1).

It is also shown that administration of niclosamide orally in the therapeutic dose, resulted in a significant increase in the activities of AST and ALT only.

Administration of combination of levamisole and niclosamide in both therapeutic doses (S.C. and orally respectively) caused a significant increase in all measured parameters (alkaline phosphatase, AST, ALT, urea and creatinine).

Histopathological findings:

Injection of rats with the therapeutic dose levamisole Hcl, niclosamide or combination of both drugs caused the following histopathological changes:

The heart of rat in gp. II showed swellig of the muscle fibers, sarcoplasmic granularity and vacuolation and focal areas of necrosis, in which the muscle fibers appear deep eosinophilic with pyknotic nuclei (Fig. 1). In gp. III, the heart showed the same myocardial degenerative changes as in gp. 11, but the sarcoplasmic vacuolation appeared more pronounced (Fig. 2). In gp. IV, the cardiac lesions appeared more extensive and were progressed to focal areas of myolysis which became sometimes infilterated with mononuclear cells (Fig. 3).

The liver in gpII, showed mild degenerative changes as swelling and in gpIII no pronounced histopathological lesions were detected, while the administration of both drugs in combination, leads to the presence of small focal hepatic necrotic areas (Fig. 4).

The kidney lesions produced in gpII and gpIV were mainly toxic tubular nephrosis specially at the corticomedullary junction. The affected tubules showed vacuolation of their epithelial lining, somtimes with complete plasmolysis and nuclear pyknosis. The degenerative changes of the tubular lining progressed in some tubules to individual cell necrosis or necrosis of whole tubular lining (Fig. 5). In gpIII, the kidney showed no specific alteration.

The spleen in gpII and gpIV showed mild histopathological changes manifested by necrosis and lysis of some lymphocytes which appear homogenous structurless bodies, in addition to congestion of the blood sinusoids in the red pulps (Fig. 6).

The spleen in gpIII not differ than of the control.

DISCUSSION

The present investigation revealed that the injection of rats with the therapeutic dose of levamisole Hcl induced a significant increase in the activities of AST, ALT and urea. These results goes side by side with the results of histopathological examination which revealed degenerative changes in the heart and kidney. *FAYEZ et al. (1983)* found similar results on the heart muscle of rats injected tetramisole Hcl. Moreover, *FARSYTH (1966)* attributed death in animals treated with tetramisole Hcl to heart failure. Also *SCHMIDT and MUELLER-ECKHARDT (1977)* mentioned that the patients treated with levamisole suffered from granulocytopenia,

thrombocytopenia and acute renal failure. The very mild hepatic histopathological changes. accompanied by insignificant alteration in the parameters of alkaline phosphatase and bilirubin confirm the results of *DUKES (1980)* who stated that tetramisole Hcl in not hepatotoxic to man and animals.

The necrosis and lysis of some lymphocytes in the spleen indicate that the levamisole Hcl affect the immune response of the treated animals, a result which in accordance with that of *EL SAIDY (1991)* who found that injection of levamisole Hcl at a dose of 36 mg/Kg b.wt. induced a significant decrease in the gamma I globulin at one day post injection and gamma 2b globulin at 7 day post injection. In this connection, *RENAUX and RENAUX (1974) and SAMPSON* et al. *(1977)* reported that administration of levamisole Hcl above the immunostimulant dose (2-3 mg/Kg b.wt.) may sppress the immune function.

The administration of niclosamide produced no effect on the kidney function, this finding is in accordance with that of *ABDELLAH and SAIF (1961)* who found no alteration in the renal function or blood counts of treated patients.

The administration of combination of the two anthelmintics, levamisole Hcl and niclosamide in the therapeutic doses caused a significant increase in all tested serum parameters (ALT, AST, bilirubin, ALP, urea and creatinine). The effect of this combination on hepatic tissue was manifested by focal hepatic necrosis and on the heart, the lesion was seen as myocardial fiber necrosis which became sometimes infiltrated with mononuclear cells. Also some degenerative changes in the kidney and spleen. These results tend to support the finding of *DUKES (1980)* that tetramisole Hcl should not be given with other hepatotoxic anthelmintics. On the other hand, our findings disagreed with that of *JONESET* et al. *(1981)* who stated that tetramisole Hcl could be given safely with other anthelmintic agents.

It could be concluded that, levamisole Hcl is cardiotoxic when given with niclosamide even if both were given in the therapeutic doses.

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Table (1)

Effect of administration of therapeutic doses of levamisole Hcl and niclosamide on serum chemistry in albino rats(M±S.E.)

Groups	Drugs mg/Kg b.wt.	AST iu/L	ALT iu/L	Bilirubin mg%	ALP iu/L	Urea mg/dl	Creatinine mg/dl
Group I	Saline	57.5 ± 3.13	17.0 ± 0.57	0.36 ± 0.02	42.13 ± 1.00	24.75 ± 1.44	4.19 ± 0.21
Group II	Levamisole 36 mg.	93.75 ± 3.98**	24.13 ± 0.58**	0.51 ± 0.03	47.13 ± 2.42	36.00 ± 1.17**	4.3 ± 0.19
GroupIII	Niclosamide 450 mg.	82.5 ± 6.75	24.00 ± 0.6	0.42 ± 0.01	43.00 ± 1.54	27.63 ± 1.38	4.28 ± 0.19
Group IV	Levamisole 36 mg. Niclosamide 450 mg.	110.0 ± 3.66**	29.25 ± 0.96**	0.57 ± 0.01**	56.75 ± 1.96**	58.75 ± 1.20**	4.88 ± 0.16**