Alexandria Journal of Veterinary Sciences 2015, 47: 148-157
ISSN 1110-2047, www.alexjvs.com
DOI: 10.5455/ajvs.199012

Adverse Effects of Nitroxynil in comparison with Levamisole on Male Rats

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Key words

Nitroxynil, Levamisole, Male Rats, Liver functions

ABSTRACT:

The present study was conducted to study the effectivness of Nitroxynil and \or Levamisole on male fertility, as well as its effects on the liver and kidney functions tests, some hematological parameters and histopathological changes. Therfore sixty mature male albino rats were used and divided into 4 equal groups. The first group: rats was kept as control and subcutaneously injected with proplyene glycol (2 ml/kg b.wt.) and distal water at dose of (2m/kg b.wt orally). The second group: was subcutaneous injection of Nitroxynilat a dose of (36mg/kg bwt). The third group: was orally administered Levamisole (7.5 mg / kg bwt.). The fourth group: was administered Nitroxynil (36mg/kg bwt .s.c)+ Levamisole (7.5 mg / kg bwt.oral) were re-administered to all groups after 30 days from the start of the experiment as the same doses previously mentioned. Five rats were killed at 2nd, 4th and 8thweeks from beginning of drug administration. The obtained results showed that administration of Nitroxynil and\ or Levamisole and their combination induced a variety of side effects on male reproduction as reduction of testes, epididymis, and accessory sex organs weights and change in sperm charactersdecreased sperm count and motility, and increase the sperm abnormalities. Liver functions tests such as serum alanine aminotransferase (ALT), aspartateaminotransferase (AST) and alkaline phosphatase (ALP) were significantly increased. Moreover, administration of Nitroxynil and\ or Levamisole induced histopathological alterations in reproductive organs, liver and kidney. Therefore, caution should be taken when using Nitroxynil and\or Levamisole in male animals.

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1-INTRODUCTION:-

Nitroxynil and Levamisole are frequently used to control nematodes, insects and fascioliasis in animals, and commonly used anti parasitic causes many side effects on the different organ of treated animals **(**Martin 1969).

The present study was designed to investigate the effect of Nitroxynil or Levamisole on male Fertility, liver and kidney functions and side effects of these drugs in rats.

Levamisole, is an antihelmintic immunomodulator belonging to a class of synthetic imidazothiazole derivatives (Raemakers, 1966). Levamisole was originally used to treat worm infestations in both humans and animals, it acts as anticholinergic receptor agonist that causes continued stimulation of the parasitic worm muscles, leading to paralysis (Keiser2014).

Levamisole acts selectively asa nicotionic acetyl choline receptors a agonist on nematode muscle cells. This causes a spastic paralysis of susceptible nematodes by selectively gating acetyl choline receptor in channels on nerve and muscle (Martin and Roberrtson, 1993)

Levamisole is rapidly absorbed following oral, intramuscular, or subcutaneous administration to several animal species. Extensive metabolism occurs with rapid excretion of drug and metabolites, equally distributed between urine and feces in rats (Adams, 1978).

Levamisole is absorbed from the gut after oral dosing and through the skin after dermal application, although bio availabilities are variable. It is reportedly distributed throughout the body. Levamisole is primarily metabolized with less than 6% excreted unchanged in the urine (Susan and Donald, 2003).

Clinical signs and lesions of Levamisole toxicity include: nausea, vomiting, increased salivation, frequent urination and defecation, colic, dizziness, headache, muscle tremors, ataxia, anxiety, hyperesthesia with irritability, clonic convulsions, depression, rapid respiration, dyspnea, prostration, collapse, hemorrhages in the sub epicardium and thalamus, enteritis, hepatic degeneration and necrosis, and splenic congestion (Hsu,1980).

Nitroxinil is highly effective against adult liver flukes (Fasciola hepatica) It is also effective against a few gastrointestinal roundworms e.g

(Bunostomumspp, Haemonchus spp, Oesophagostomum spp, and Parafilaria bovicola) as well as against myiases but it is not effective against rumen flukes (Paramphistomum spp), other roundworms such as lungworms e.g (Dictyocaulus spp) or eye worms e.g (Thelazia spp), tapeworms and other external parasites (Junquera, 2007).

Nitroxynil is slowly absorbed but well absorbed from GIT. peak plasm concentration is attained within 5hours and Nitroxynil attains lower concentration in tissue than plasma and plasma protein is binding 97-98% in cattle and sheep (Kingsley,2014)

The high dose of Nitroxynil may cause blindness and signs of uncoupled oxidative phosphorylation, hyperventilation, hyperthermia, convulsions tachycardia and ultimately death(Andrews, 2008).

However the commonly used anti parasitic causes many side effects on the different organs of treated animals (Martin, 1969) and the adverse effects of administration of nitroxynil and /or levamisole are rather scarce. The present study was designed to investigate the effect of nitroxynil and/ or levamisole on male fertility, liver and kidney functions, blood picture and histopathological findings in some organs in male rats.

Concurrent administration of Nitroxynil with nematocide (e.g levamisole) or with clostridial vaccine have ill effects on liver, and kidney functions and blood picture (Kingsley, 2014).

2-Materials and Methods

2.1. Drugs

a.Levamisole

Levamisole Hydrochloride ph.Eur.**(Levacide injection)®**

Manufactured by: Norbrook laboratories limited, Newry,Co.Down, BT 35 6 jp.

b-Nitroxynile (Dovenix)® Produced by: Merial Co.France.

2.2 Experimental design:

The studies were carried out on 60 albino male rats of 130-170g body weight each and a about 140-160 days age . the animals were purchased from the Medical research Institute of Alexandria University.The present work was conducted to evaluate the effect of Nitroxynil or Levamisole and their coadministration in male rats on fertility, liver and kidney function, blood picture and histopathological findings in some organs.

The animals were divided equally into 4 groups each of 15 rats as follows:

The first group: was given propylene glycol 2 ml/kg bwt subcutaneous injectionand distal water at dose of (2m/kgb.wt orally).

The second group: was given(7.5 mg/kg bwt) of levamisole oral

The third group: was given Nitroxynil (36 mg/kg bwt s.c)

The fourth group: was given of Levamisole (7.5 mg/kg bwt oral)+ Nitroxynil (36mg/kg bwt .s.c). Dose of each drug was calculated to rats according to **Paget and Barnes (1964).**

All drugs were re-administered to all groups after 30 days from the start of the experiment as the same doses previously mentioned. Five rats from each treated and control group were killed after 2nd, 4th, 8thweek from the beginning of drug administration . Blood, body organs and epididymal contents were obtained from treated and control rats.

2.3. Blood sampling:

Two blood samples from each control and treated rats were taken before sacrifying them from orbital plexus (inner canthus of the eye) under light ether anaesthesia using heparinized hematocrite tube. One sample was taken with EDTA for blood picture while the other sample was taken without anticoagulant and left to clot at room temperature then centrifuged for 15 min at 3000 r.p.m to obtain clear serum. The sera were identified and stored in deep freezer at −20ºC till used for biochemical analysis.

2.4. Fertility studies:

Rats were sacrifised by decapitation and the epididymal content of each rat was

taken by sharp cutting of the tail of epididymes and squeezed gently on sterile glass watch to estimate the progressive motility, sperm cell count and sperm abnormalities according to the method described by Berdan and Fuquay (1980).

a- Sperm progressive motility and abnormalities:

A clean dry slide was placed on heated stage microscope and allowed to

warm. A drop of semen was placed on the clean dry slide, mixed with two drops of saline using glass rod. Uniform mixture must be prepared to estimate accurate determination. The progressive motility percentage was estimated and recorded, then immediately two equal drops of Eosin-Nigrosine stain were added to the diluted semen and mixed well then the film was spread on the slide. Three hundred sperms

were observed under high power lens and the percentage of abnormal sperms was estimated and recorded.

b-Epididymal sperm count:

For counting epidiymal sperm, a hemocytometer and a pipette were used. A drop of cauda epididymal content of each control and treated rats was withdrawn up to mark 0.1 and the pipette was then filled up to the mark 101 by the sodium bicarbonate solution 5% for breaking up the mucus droplets in the hemocytometer pipette. The content of pipette was mixed by holding the ends of pipette between the thumb and the index fingers and shaking it vigorously. The cover slip was placed over the counting chambers and the tip of the pipette was dried by fingers. Few drops of fluid were discarded, then a small amount of diluted semen was drawn under the cover by the capillary action.

2.5. Weight of Internal Body Organs:

Aftar collection of the blood samples and epididymal sperm examination, testes , accessory sex organs [prostate and seminal vesicle] ,epididymis , were dissected out , grossly examined and weighed . The index weight [I.W] of each organ was calculated as described by **Matousek (1969**).

$$\text{Index weight[I.W.]} = \frac{organweight}{bodyweight} \times 100$$

2.6. Biochemical studies:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was activity were measured colourimetrically according to the method described by **Reitman and Frankel (1957)** and Alkaline phosphatase activity was measured according to the method described by **Kind and King (1954)**. Total protein was measured by the colourimetric method as described by **Doumas et al. (1971).** Serum albumin level was determined colourimetrically according to the method described by **Doumas et al. (1971).**Serum globulin level was determined by subtracting the albumin value from total protein value of the same sample as described **Coles (1974).** Serum urea activity was measured by the enzymatic colourimetric method as described by **Coulomb and Farreau (1963)**.Serum creatinine activity was measured by the colourimetric kinetic method as described by **Husdan and Rapoport (1968)**

2.7. Hematological studies:

a-Haemoglobin concentration(Hb):was determined according to the method described by **Benjamin (1978)** by using Sahlis haemocytometer .

b-Pack cell volume (PCV)percent: Each blood sample was mixed then the microheamatocrite tubes were filled by capillary action and the opposite end of the tubes were sealed by especial clay(**Dacie and lewis , 1984).**

c-Erythrocytic count : Each blood sample was gently mixed then diluted by using Haym's solution for erythrocytic count in respective blood count pipette. Double improved Neubauer haemocytometer was used in the count (**Dacie and lewis , 1984).**

d-Total leukocytic count : Each blood sample was gentiy mixed thendiluted by using Turk,s solution for total leukocytic count in respective blood count**(Dacie and lewis , 1984).**

2.8. Histopathlogical studies:

Following complete necropsy of the experimental male rats, small fresh specimens from liver, kidney, testes, epididymis, accessory sex organs were collected and rapidly fixed in 10% formalin solution for at least 24h . after that ,these specimens were processed through the convential paraffin embedding technique [dehydration in ascending grades of ethyl alcohol , clearing in different changes of xylene and embedding in different changes of melted paraffin wax at 60c].paraffin blocks were cut by microtome into 5 microns ,thick sections which were stained by Haematoxylin and Eosin [H.E] ,according to the method described by Harries (1989) and were examined.

2.9. Statistical analysis

Statistical analysis was performed using the SAS computer program **(SAS, 2002)**

3-RESULTS

3.1. Fertility studies:

1- Reproductive organs index weight:

It was found that subcutaneous administration of Nitroxynil (36mg/kg bwt) or oral administration of Levamisole(7.5mg\kg .b.wt) alone and in combination. There was a significant decrease in weight of testis, epididymis and

Accessory sex organs in all groups at 4^{th} and 8^{th} weeks of the experiment as compared with control (Table 1).

2- Sperm motility%, sperm count, sperm abnormalities%

The obtained results showed that subcutaneous administration of Nitroxynil (36mg/kg bwt) or oral

administration of Levamisole (7.5mg\kg. b.wt) and their interaction at different periods of experiment induced a significant decrease in the progressive sperm motility % in all groups at 2nd ,4th and 8thweeks of the experiment as compared with control group. However, there was a significant decrease in sperm count in all groups at the4th and 8th weeks of the experiment as compared with control group. Moreover, there was a significant increase in sperm abnormalities in all groups at the 4th and 8th weeks of the experiment as compared with control

3.2. Biochemical studies:

There was a significant increase in some liver enzyme marker after subcutaneous administration of Nitroxynil (36mg/kg bwt) or oral Levamisole (7.5 mg/kg bwt) or their interaction. The obtained data showed that there was a significant increase in serum ALT and AST in all groups at 4th and 8ththe weeks of the experiment as compared with control group. The increase was more pronounced at 8th week of the experiment. Moreover, there was a significant increase in ALP in all groups at the 4th and 8thweeks of the experiment as compared with control group (Table 3).

Also, There were a significant increase serum urea, and creatinine levels in all treated groups at the2nd ,4th and 8th weeks of the experiment as compared with control group (Table 4). While the treatments did not induce any alteration in serum total protein and albumin levels allover experimental periods as compared with control group.

Moreover, there were a significant increase in serum globulin at the 2nd, 4th and 8th weeks of the experiment as compared with control group (Table5).

3-3- Hematological studies {Red blood corpuscles (RBCs), white blood cells (WBCs) , haemoglobin (Hb) Concentration (g%) and packed cell volume percent (PCV%)}

There was a significant increase in RBCs, WBCs count, Hb content and the PCV percent in all treated groups at the 4th and 8th weeks of the experiment as compared with control group (Table 6).

Table1. Effect of subcutaneous administration of Nitroxynil (36mg/kg bwt) or Levamisole(7.5 mg/kg b.w oral) and their interaction at interval of 30 days on the index weight of reproductive organs in adult male rat at different periods from beginning of drugs administration

parameters	testis index weight			Epididymis index weight			Accessory sex glands index wight		
Time Group,	2nd week	4th week	8th week	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	A 1.20 ±0.01	A 1.21 ±0.04	A 1.20 ± 0.01	A 0.46±0.01	A 0.60±0.03	A 0.50 ±0.03	AB 0.56±0.02	A 0.67±0.03	A 0.62± 0.02
Nitroxynil	A 1.17±0.02	B 1.13±0.01	B 1.07 ±0.01	A 0.46±0.01	B 0.47±0.03	B 0.42±0.02	A 0.59±0.01	B 0.57±0.02	B 0.49±0.02
Levamisole	A 1.15± 0.02	B 1.11± 0.02	B 1.09 ± 0.01	A 0.42±0.01	B 0.47±0.02	B 0.42± 0.01	A 0.59±0.01	B 0.54±0.02	B 0.52 ±0.02
Nitroxynil+ Levamisole	A 1.17± 0.01	B 1.12± 0.01	B 1.10 ± 0.02	A 0.44±0.02	B 0.40±0.01	B 0.40 ±0.02	B 0.53±0.01	B 0.52±0.01	B 0.48± 0.01

-Values are expressed as Means±S.E n=5

Means within the same column carrying different letters are significantly different ($p<0.05$)

Table 2. Effect of subcutaneous administration of Nitroxynil (36mg/kg bwt) or Levamisole(7.5 mg/kg b.w oral) and their interaction at interval of 30 days on fertility parameters in adult male rat at different periods from beginning of drugs administration

parametes	Sperm motility (%)			sperm count (×106/ml)			Sperm abnormalities (%)		
Time Group,	2nd week	4th week	8th week	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	A 92.50±1.44	A 91.67±1.67	A 91.67±1.66	A 195.00 ±2.89	A 221.67±10. 93	A 205.00±2.88	A 25.25±2. 75	B 19.75±0. 63	B 24.75±1.80
Nitroxynil	B 77.50±1.44	B 75.75±1.49	B 75.00 ±2.04	A 164.20 ±6.43	B 166.25±6.57	B 161.25±6.57	A 29.40±0.40	A 28.60±0.60	A 31.20±0.58
Levamisole	B 75.20±1.28	B 74.20±1.66	B 72.60±1.12	A 176.00±1.97	B 166.00±5.10	B 161.8 0±4.5	A 29.00±2.30	A 28.00±1.18	A 32.00±0.45
Nitroxynil+ Levamisole	B 75.60±1.17	B 73.60±1.86	B 73.25±2.36	A 172.40±2.69	B 165.0 ±7.25	B 167.5 ± 3.23	A 30.40 ±0.7	A 29.60±0.75	A 32.40±0.75

-Values are expressed as Means±S.E n=5
Means within the same column carrying different letters are significantly different ($p<0.05$)

Table 3. Effect of subcutaneous administration of Nitroxynil (36mg/kg bwt) or Levamisole (7.5 mg/kg b.w oral) and their interaction at interval of 30 days on liver enzymes level in adult male rat at different periods from beginning of drugs administration.

parameters	ALT (U/L)			AST (U/L)			ALP (U/L)		
Time Group,	2nd week	4th week	8th week	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	A 31.67 ±0.88	B 35.00±1.47	C 35.00±1.47	A 23.33± 2.40	B 37.0 0±0.32	C 35.00 ±1.47	A 196.25±5.54	B 159.25±3.50	B 148.8 0±6.76
Nitroxynil	A 36.50±0.65	A 51.80±0.90	B 69.00±8.73	A 27.25±2.46	A 44.08±1.87	A 59.60 ±8.45	A 196.40±2.74	A 185.60±5.28	A 185.80±0.06
Levamisole	A 33.40±1.78	A 51.40±1.12	AB 79.80±3.61	A 25.80±1.90	A 42.20±0.97	B 46.32±14.14	A 189.40±3.50	A 185.60±7.64	A 191.66±6.29
Nitroxynil+ Levamisole	A 34.92±2.16	A 59.80±0.92	A 91.00 ±0.63	A 22.50±3.17	A 41.0 0±1.15	A 55.80 ±5.85	A 193.80 ±5.54	A 187.6 0±8.58	A 193.25 ±9.04

-Values are expressed as Means ±S.E n=5
Means within the same column carrying different letters are significantly different ($p<0.05$)

Table 4. Effect of subcutaneous administration of Nitroxynil (36mg/kg bwt) or Levamisole(7.5 mg/kg b.w oral) and their interaction at interval of 30 days on Urea (mg/dl) and creatinine(mg/dl) level in adult male rat at different periods from beginning of drugs administration

Parametes	Urea (mg/dl)		Creatinine(mg/dl)			
Time Group,	2nd week	4th week	2nd week	4th week	8th week	8th week
Control	B 23.45 ±1.06	B 24.43 ± 0.72	B 1.9 0 ± 0.23	B 1.9 6 ± 0.58	B 1.9 5± 0.34	B 23.28 ±0.16
Nitroxynil	A 32.95 ±1.49	A 36.85 ±4.7	A 3.13 ± 0.09	A 3.06± 0.15	A 3.91± 0.23	A 31.74 ±0.09
Levamisole	A 34.22 ±1.42	A 42.54 ±1.49	A 3.20± 0.45	A 3.90 ± 0.05	A 3.70± 0.30	A 31.49 ±0.17
Nitroxynil+ Levamisole	A 32.86 ± 0.80	A 42.22 ±1.46	A 3.98 ± 0.21	A 3.37 ± 0.24	A 3.78± 0.34	A 32.88 ± 0.10

-Values are expressed as Means ±S.E n=5
Means within the same column raw carrying different letters are significantly different ($p<0.05$)

Table 5. Effect of subcutaneous administration of Nitroxynil (36mg/kg bwt) or Levamisole(7.5 mg/kg b.w oral) and their interaction at interval of 30 days on total protein(g/dl), albumin(g/dl) and globulin (g/dl) level, in adult male rat at different periods from beginning of drugs administration.

parameters	Total protein (g/dl)		Albumin (g/dl)			Globulin (g/dl)			
Time Group,	2nd week	4th week	2nd week	4th week	8th week	8th week	2nd week	4th week	8th week
Control	A 5.01 ± 0.09	AB 6.0 0± 0.40	C 0.35 ± 0.03	B 0.27 ± 0.09	B 1.3 0± 0.11	A 6.37 ± 0.22	AB 4.4 6± 0.38	AB 5.66 ± 0.6 2	A 2.9 0±0.51
Nitroxynil	A 5.64 ± 0.44	B 5.17 ± 0.35	B 0.81 ± 0.02	A 0.43± 0.04	A 1.6 5± 0.12	A 6.63 ± 0.25	B 3.33 ± 0.34	B 4.1 8± 0.49	A 2.53 ± 0.20
Levamisole	A 5.14 ± 0.25	AB 5.88± 0.73	B 0.8 2 ± 0.01	A 0.42 ± 0.01	A 1.6 2± 0.09	A 6.0 4± 0.18	B 3.53 ± 0.15	B 4.20 ± 0.21	A 2.20± 0.15
Nitroxynil+ Levamisole	A 6.0 6± 0.3	A 7.32 ± 0.25	A 1.16±0.14	A 0.47± 0.02	A 1.6 4± 0.08	A 6.33 ± 0.28	A 5.5 8± 0.35	A 6.02 ± 0.3 7	A 2.8 5± 0.27

-Values are expressed as Means ±S.E n=5

Means within the same column carrying different letters are significantly different ($p<0.05$)

Table6. Effect of subcutaneous administration of Nitroxynil (36mg/kg bwt) or Levamisole(7.5 mg/kg b.w oral) and their interaction at interval of 30 days on hematological parameters count in adult male rat at different periods from beginning of drugs administration.

parametes	PCV %			RBCs count (×106/cmm)			Hb (g/dl)			WBCs count (×103/cmm)		
Time Group,	2nd week	4th week	8th week	2nd week	4th week	8th week	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	A 33.07 ±1.07	B 31.70± 1.46	B 34.13± 2.12	A 6.86 ±0. 55	B 5.77± 0.37	B 3.76± 0.37	B 13.78 ±0.60	B 14.27± 0.41	B 10.90 ± 0.69	A 7.55± 0.84	B 7.30± 1.19	B 7.43± 0.67
Nitroxynil	A 37.88 ±0.88	A 33. 58± 2.13	A 54.55 ±5.13	A 6.60± 0.46	A 6.43± 0.43	A 5.7 9± 0.46	B 13.78± 0.60	A 15.76 ±0.43	A 12.29 ± 0.75	A 7.56± 0.84	A 9.30± 0.54	A 10.30± 0.29
Levamisole	A 33.68 ±1.44	A 36.02± 1.46	A 55.60 ±5.38	A 6.91± 0.41	A 7.05 ± 0.46	A 5. 77 ±0.48	B 15.10±0.67	A 15.80± 0.18	A 13.36± 1.03	A 7.12± 0.64	A 9.80± 0.87	A 10.36± 1.74
Nitroxynil + Levamisole	A 36.70 ±2.34	A 34.58 ±1.86	A 55.60± 5.37	A 7.50 ± 0.42	A 6.05± 0.22	A 5.7 7 ± 0.48	B 14.80±0.35	A 15.76 ± 0.4 6	A 13.7 8± 0.32	A 7.58± 0.83	A 9.04± 0.36	A 10.48± 0.39

-Values are expressed as Means ±S.E n=5

Means within the same column carrying different letters are significantly different ($p<0.05$)

4. Histopathological examination:

The microscopical examination of testicular tissue of groups treated with (Nitroxynil (36mg/kg b.wt s.c) or Levamisole (7.5 mg/kg b.w oral) and interacted group showed congestion blood vessel, hypo-spermatogenesis, interstitial edema and seminiferous tubules at the 4th and 8ththe weeks of the experiment (Fig1).

Regarding to prostate gland in all treated groups there were secretions, congestion, edema and infiltration with some pyknotic nuclei and aggregation at 4th week and 8th week of experiment (Fig2). Moreover, the seminal vesicles of rats treated with (Nitroxynil (36mg/kg b.wt s.c)or Levamisole (7.5 mg/kg b.w oral) and interacted group (36mg/kg b.wt.s.c) there was secretion, glandular acini separated with interstitial edema and congested blood vessels at the 4th and 8ththe weeks

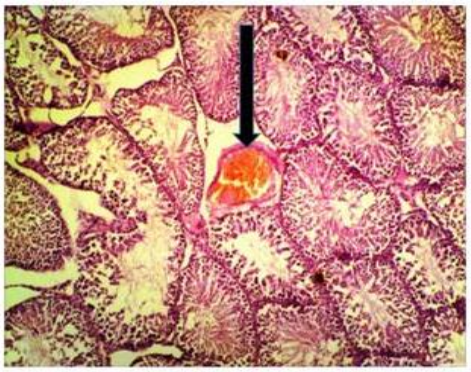
of the experiment (Fig3).The epididymis of rats of all treated groups showed spermatozoal contents, congestion, edema and fibroplasias , infiltration aggregation, interstitia lymphocytic, dilation and epididymal tubules separated by area of fibroplasia and numerous congested blood vessels all over the experimental period (Fig4). The microscopical examination of the livers in all treated groups revealed lymphocytic aggregation, vacuolation and congested portal blood vessels with few lymphocytic infiltrations in addition to intra-lobular congestion and lymphocytic reaction at the 4th and 8ththe weeks of the experiment (Fig5). The examined kidneys of rats treated with (Nitroxynil (36mg/kg b.wt s.c)or Levamisole (7.5 mg/kg b.w oral) and interacted group revealed congestion (cortical or medullary) , hemorrhages, lymphocytic infiltration,Tubular dilation and Tubular cast and excess of luminal casts in the cortical tubuleat the 8ththe weeks of the experiments (Fig 6).

4. DISCUSSION:

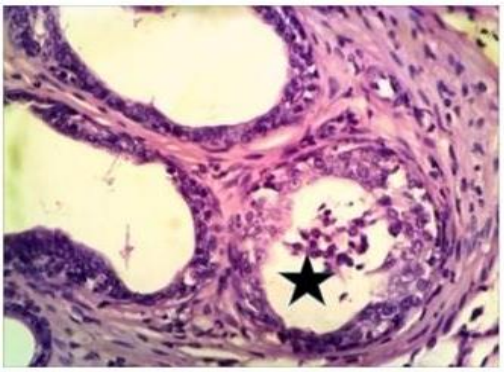
The present study investigated the possible adverse effects of the subcutaneous injection of Nitroxynil (36mg \kg B.wt) and \or Levamisole (7.5 mg \kg B.wt. orally) either alone or in combination in mature male rats.

Measurements were made at different periods from the onset of drugs administration to follow up the induced effects of the drugs on some reproductive organs weight, semen characters and hepatic and renal function tests ,blood picture, and extended to some histological changes in some organs of the mature male rats.

The results of the present study demonstrated a significant reduction in testes, epididymis and accessory sex glands weight after4th and 8th weeks of the experiment. sperm characters, the obtained results revealed that, there was a significant reduction in Sperm count in all treated groups at 4th and 8thweeks of the experiment. Also, there was a significant reduction in the progressive sperm motility % in all treated groups at 2nd 4th and 8th weeks of the experiment.



Fig(1). Testes of rat administered Nitroxynil (36mg \ kg B.Wt) and killed 4weeks post administration showed hypo-spermatogenesis, interstitial congestion interstitial edema and seminiferous tubules with nearly normalspermatogenesis, while the interstitial blood vessel appeared severely congested (arrow) H&E, X 160.



Fig(2). Prostate gland of rat administered Levamisole(7.5 mg/ kg b.w oral)and killed 8weeks post administration showed secretions,Luminal cellular , congestion , edema , interstitial lymphocytic infiltration aggregation and secretory epithelium with some pyknotic nuclei and luminal contents of desquamated cells (asterisk). H&E, X 400.

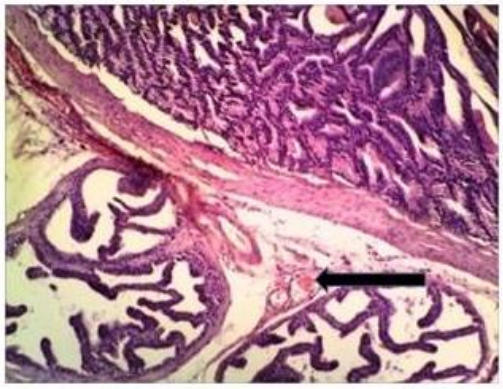


Fig (3). Seminal vesicle of rat administered Nitroxynil (36mg \kg B.Wt sc)and killed 8weeks post administration showed secretion, congestion, edema glandular acini separated with interstitial edema and congested blood vessels (arrow). H&E, X 160

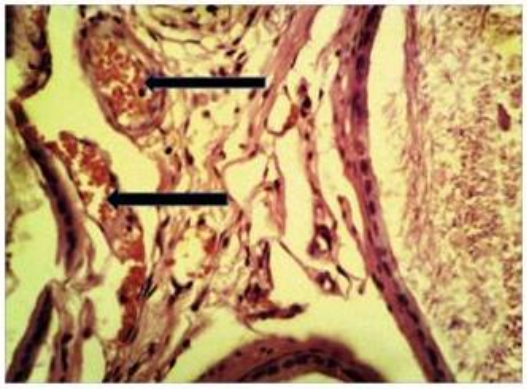


Fig (4). Epididymis of rat administered Nitroxynil (36mg \kg B.Wt sc) +Levamisole(7.5 mg/kg b.w oral) and killed 8weeks post administrations howedepididymal tubules separated by area of fibroplasia and numerous congested blood vessels (arrows). H&E, X 400.

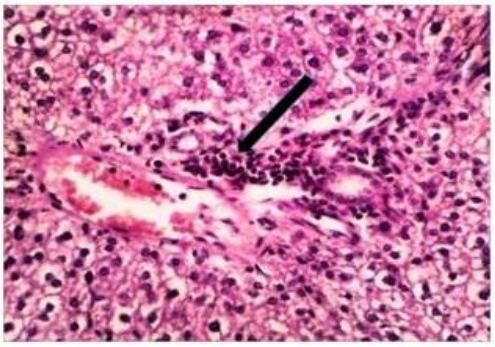


Fig (5). Liver of rat administered Nitroxynil (36mg \kg B.Wt sc) +Levamisole(7.5 mg/kg b.w oral and killed 4weeks post administration showed congestion, lymphocytic aggregation, vacuolation and congestion of the portal blood vessel and some perivascular lymphocytic aggregations (arrow). H&E, X 400

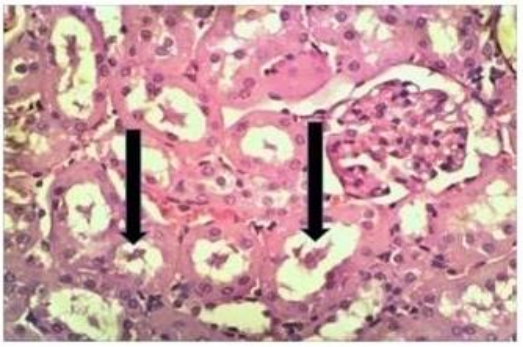


Fig (6). Kidneys of rat administered Nitroxynil (36mg \kg B.Wt sc) and killed 8weeks post administration showed congestion (cortical or medullary), hemorrhages, lymphocytic infiltration, tubular dilation and tubular cast and excess of luminal casts in the cortical tubules (arrows). H&E, X400.

sperm characters, the obtained results revealed that, there was a significant reduction in Sperm count in all treated groups at 4th and 8thweeks of the experiment . Also, there was a significant reduction in the progressive sperm motility % in all treated groups at 2nd 4th and 8th weeks of the experiment.

Moreover, there was a significant increase in total sperm abnormalities % in all treated groups at 4th and 8th weeks in the experiment. The observed decrease in male rats fertility are in agreement with those obtained with El-Desouki et al. (2011) found that Levamisole (7.5mg\kg b.wt.orally) caused a significant decrease in the sperm cell concentration ,progressive motility and alive sperm percentages, while total sperm abnormalities was significantly increased. Moreover, Cordero (1999) found that threapeatic dose of Levamisole (7.5mg\kg b.wt.orally) in rams caused significant ($P < 0:05$) decreases in the sperm cell concentration and progressive motility sperm.

Also these results agree with those recorded by Souria et al.(2007) who found (40mg\kg b.wt.s.c) Nitroxynil in farm animals, caused a signification decrease in sperm count , sperm production ,sperm cell , and decreases of fertility.

These findings could be attributed to the reported histological changes in testes and epididymis which in form of odeam, congested of blood vessels, hypospermatogenesis, dilation fibroplasias and lymphocytic on epididymis in Nitroxynil and Levamisole treated groups all over the experimental period. These lesions were also in intrecation group.

Histopathological findings in prostate gland represented as screation, congestion, edema, lymphocytic and aggregation in all treated groups the lesions in seminal vesicles congestion and edema in all treated groupsallover the experimental period .

In the current study, it was found that administration of Nitroxynil (36mg\kg b.wt.s.c) and\or Levamisole (7.5mg\kg b.wt.orally) produced significant increase in WBCs, RBCs count, PCV% and Hb concentration in all tread groups after 4th and 8thweeks of experiment as compared with control group.

These results are agree with those recorded by Caple et al.(1978) who found that treatment of elephants with Nitroxynil(10mg\kg b.wt.s.c) induced a significant increase in Hb concentration, PCV,W BCs and RBCs count .Moreover, Mohamed et al.(2013) reported that, administration of levamisole at (7.5 mg\kg .b.wt.orally) to rabbits induced a significant increase in RBCs,WBC counts.

On the other hands these results were disagree with those reported by Cokce et al.(2004) who found that injection of dogs with Levamisole(25mg\kg.b.wt orally) induced decrease in RBCs, PCV and Hb concentration.

In the current study, the activities of ALT, ALP and AST were significantly increased in all treated groups after the 4th and 8th weeks of the experiment as compared with control group, These results are supported by the damage of liver in histopathological findings reported in the present study. When liver damage occurs, the cell membranes become permeable or the cell wall may rupture, so the ALT, ALP diffuse into the blood stream and increased levels are found in the circulatory blood (Doxy 1971; Hoe and Wilkinson, 1973).

These results are supported by the findings reported by Dawson et al. (2009) they found that injection of Nitroxynil (10.2mg\kg B.wt .s.c) in cattle,

significantly increase AST and ALT above the rang 8and 11 week after injection, Atessahin et al .(2004) they recorded that administration of therapeutic dose of Levamisole (7.5mg\kg b.wt.orally) in sheep significantly increased the serum levels of ALT, AST and ALP compared with the control levels .

Our results disagree with those obtained byMohumed et al. (2013) who found that treatment of rabbits with oral dose of Levamisole (2.5mg\kg.b.wt) significantly decreased the serum levels of ALT, AST. These results are confirmed by the reported histopathological findings in this study, which revealed the hepatic change in nitroxynil treated group in from of congestion of the portal blood vessel, hapatocytic, hemorrhages and perivascular lymphocytic aggregation on 4th week and increased in distribution on the8th week. The hepatic changes in levamisole treated group in form of excess portal lymphocytic, aggregation, congestion, hemorrhages, vacuolation and lymphocytic on the4th week, which increased in distribution on the 8th week.

In nitroxynil and levamisole treated group the hepatic change in form of excess of the hepatocytic vacuolation with the vascular congestion and perivascular, lymphocytic aggregation on4th week with hemorrhages during on the8th week. The present study did not produce any alteration in serum total protein, albumin allover experimental periods as compared with control group.

Our results agree with those reported by Martinez et al. (2010) found that S.C injection of sheep with Nitroxynil (10mg\kg.b.wt.) not produced significant effect in the serum total protein and albumin.

Our results disagree with those obtained by Shadia et al. (2011) as they recorded that administration of Levamisole (2.5mg\kg.b.wt orally) in rabbits significantly increased the serum levels of total protein and albumin.

In the current study the levels of globulin were significantly increased in all treated groups. Our result are agree with those reported by Mohamed et al. (2013) who found that administration of levamisole (2.5mg\kg.b.wt orally) in rabbits significantly increased the level of globulin. Also, Fakhry et al. (2011) found that administration of levamisole (2.5mg\kg.b.wt orally) in rabbits significantly increased the levels of serum globulin. Also, Caple et.al(1978) found that injection of elephant with nitroxynil at a dose of (10mg\kg.b.wt) s.c induced significant increase in globulin.

In the current study the levels of serum urea and creatinine were significantly increased in all treated groups.

Our results agree with those reported by Atessahin et al. (2004) who found that Levamisole with therapeutic dose in sheep (7.5mg\kg.b.wt)orally induced significant increase in serum level of creatinine and urea.

Our results are incompatible with those reported by Fakhry et al.(2011)who found that administration of levamisole (7.5mg\kg.b.wt orally) in rats induced significantly reduction in serum levels of creatinine. Also, Hussain (1999) found that injection of rats with nitroxynil at a dose (36 mg\kg.b.wt) s.c induced significant reduction in serum levels of urea and creatinine.

The gross renal damage goes hand by hand with the histopathological findings. In Nitroxynil tread group there was congestion (cortical or medullary), hemorrhages, lymphocytic infiltration, Tubular dilation and Tubular cast and excess of luminal casts in the cortical tubule at 8ththe weeks of the experiment. While in Levamisole tread group there was excess of the casts and debris inside the lumina of the medullar tubules, Tubular dilation, congestion (cortical or medullary) and tubular cast at 4th and 8thweeks of the experiment, while in nitroxynil and levamisole treated group there was the casts formations and luminal debris associated with intertubular lymphocytic infiltration, Tubular dilation and congestion (cortical or medullary) at 4th and 8thweeks of the experiment were recorded.

5. CONCLUSION

It could be concluded that administration of nitroxynil and /or Levamisole induced a variety of adverse effects, represented by certain fertility troubles , alteration in blood picture. Moreover, the drugs induced some degree of hepatic and renal damage. So, we should use nitroxynil and Levamisole in male animals used in artificial or natural insemination to avoid its possible adverse effects on the fertility of farm animals

6. REFERENCES:-

Adsm, S.J.G. 1978**.** Pharmacokinetics of levamisole .J. Rheumatol Suppl. 4:137-42.

Andrews .2008 . Bovine Medicine Diseases and Husband dry of cattle. Chapter 60, Pag1024.

Atessahin, A., Karahan, I., Pirinccil.2004. Effectes of therapeutic and toxic doses of levamisole on thyroid hormones and some biochemical parameters in sheep .Cell Biochem. 22 (5):281-28.6

Bearden, H., Fuquay, J. 1980. Applied Animal Reproduction . Reston Publishing Co., Inc. Reston , Virginia , P. 158- 160.

Benjamin, M. M. 1978**.**Veterinary clinical pathology .3rd ED. The lowa State Univ . Press, Ames ,USA.

Caple, I.W., Jainudeen, M.R., Buick, T.D. ,Song, C.Y. 1978. Some clinico-Pathologic findings in elephants (Elephas maximus) infected with Fasciola jacksoni. J wild Dis. 1978 Jan; 14(1):110-115

Coles, E .H. 1974. Veterinary Clinical Pathology .1st Ed .W.B. Saunders Co. Philadelphia ,London , Toronto. P.21-213. Cordero, del., Campillo, M. 1999. Parasitol. Vet.. McGraw-Hill- Interamericana de Espan˜a, S.A.U.; Madrid

Coulomb, J.J. Farreau, L. 1963. A new simple semi-micro method for colourimetric determination of urea .Clin .Chem . 9:102.

Dacie, J.V., Lewis, S.M. 1984. Practical Haematology . 6th Ed ., ELBS and Churchill Livingstone, London ,UK

Dawson, K., Fitzgibbon, C.C., Martin, P.J .2009. Efficacy of an injection combination an the lmintic (nitroxynil+ clorsulon+ ivermectinctin) against early immature fasciola hepatica compared to triclabendazole combination flukicides given orally or topically to cattle. Vet. Parasitol. 162 (3-4): 278-284.

Doumas, B.T., Carter, D.D., Peters, J., Schaffer, R.A. 1975. A candidate reference method for determination of total protein in serum. 1. Development and validation. Clin. Chem. 27:1642-1643.

Doxy, D.L. 1971. Veterinary Clinical Pathology 1st Ed.London, W.B. Saunders Company PP . 556.

EL-Desoki.2001. Effect of levamisole on male fertility and its interaction with P-glycoprotein inhibitor (verapamil) in rats. Environmental Toxicology and Pharmacology, 26: 206–211.

EL-sayed,E.M., Hamd, I.R. 1997**.** Effect of Anagallis Arvensis Against natural in festation of sheep. Alex.j.ve. Scil13 (2):101-112.

Fakhry,S.S., Osma,B., Ahmed,N. 2011.Biochemical and pathological studies on effects of levamisole and chlorambucil in rat vet ital 2011 jan –Mar 47(1):89-95

Harries, M. L.1989.Some studies on parasitic gastroenteritis in sheep . Oxford Univ. Press, New York , Toronto , P .33-48 .

Hoe, C. M.Wilkinson,J.S. 1973.liver function. A review. Aust. Vet. J. 49:136.

Hsu, W.H. 1980.Toxicity and drug interaction of levamisole. J. Am. Vet. Med. Assoc. 15: 176 1166-1169.

Husdan, H., Rapoport, A. 1968. Estimation of the creatinine by the Jaffe reaction. A comparison of three methods .Clin .Chem. 14; 222.

Hussain, S. M. 1999. A study on blood parameters and comparative efficacy of different fasciolicidal drugs against fascioliasis in cattle. MSc., Thesis College vet. Sci.

Junquera, J. 2007. Parasites of Dogs, cat, Livestock : Biology and control. Last updated on februar J. vet. PP 3455

Keiser, J. 2014. Effect of Current drug soil- transmitted helminth infection Systematic review and meta-analysis. journal ListPLoS Negl Trop Disv. 8(4).

Kind, P. R., King, E. G. 1954. Colourimetric determination of alkaline phosphatase, Journal of Clinical Pathology, 7: 322

Kingsley, E. 2014.Essential Drug Date for Rational Therapy in veterinary Practice. Page 300.

Martin,J.T.,Holloway,D.A., kins, Je., Evans,R.A. 1969. The determination of nitroxynil in experimental animals and in meat by polarography**.** Proc. Soc. Anal. Chem.**,** 6**:** 143-149.

Matousek, J. 1969. Effect on spermatogenesis in guinea pigs, rabbits and sheep after their immunization with sexual fluid of bulls. J. Report. Fert. 19:63-72.

Martin, R.J., Robertson, A.P .1993. Mod of action and Pyrantel, anthelmintic resistance, E153 and Q47. Parasitology . 134 (Pt8):1093-1014.

Martinez, V. M., delrosario, F. M., Fernandez, P. N., Castanon .,Vazquez, F.A.. 2010. Efficacy of nitroxynil against Fasciola hepatica resistant to triclabendazole in a naturally infected sheep flock. parasitol Res. 107 (5):1205-211.

Mohamed, E. E., Nany, N.E. 2013. Comparative study on the effect of fucoidan and levamisole on selective Biochemical and Hematological parameters in Heat stress in rabbits. Int. J. Pharmaceut. Medical research (1). Woar Journals Page 1

Paget, G.E., Barnes, J.M. 1964. Evaluation of Drug Activities , Toxicity Testes . Pharmacometrics . Academic Press, London .New York.

Raey makers, A.H. 1966. Levamisole is an antihelmintic. J. Med. Pharm. Chem. 9:545-551.

Reitman, S. , Frankel ,S. 1957.A colourimetric method for the determination of serum

glutamate oxalacetic acid and pyruvic acid transaminase .Am. J. Clin. Pathol. 28:56-63.

SAS, 2002. Statistical Analysis system. User's Guide: Statistics. SAS Institute , Cary, North Carolina.

Shadia, A. R., Fetouh, E.H., Riham, M., El-Rashidy, b .,Omaima, M. S. 2011 biochemical andPathological effects of closantel and levamisole in female rabbits Benha Vet. Medical Journal, 22 (2):136-144.

Souria, M., Entessar, E. 2007. Effect of crushed nitroxynil and closantel on physical and microbica cell ,. National research center , Egypt.

Susan, K., Donald, C. 2003. Levamisole Elephant care international. Repote. Vet. 20-59.