Adverse Effects of Ivermectin in comparison with Rafoxanide on Male Rats

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Abstract
The present study was conducted to evaluate the effect of Ivermectin and/or Rafoxanide on male fertility, as well as their effects on the liver and kidney functions tests, some hematological parameters and histopathological changes. Sixty mature male rats were used and divided into 4 equal groups. The first group was kept as control and subcutaneously injected with propylene glycol (2ml/kg b.wt. s,c) and were given distilled water at dose of 2ml/kg b.wt. orally. The second group was injected with Ivermectin at a dose of (0.56 mg/kg bwt s.c). The third group was given Rafoxanide (7.5 mg / kg bwt orally). The fourth group was injected with Ivermectin (0.56mg/kg b.w. s,c) together with (Rafoxanide 7.5mg/kg b.w oral). The treatment regimen was repeated to all groups after 3 days from the start of the experiment. Five rats from each treated and control group were killed after 2, 4, 8 weeks from the beginning of drug administration. The obtained results showed that administration of Ivermectin and/or Rafoxanide induced a variety of side effects on male reproduction as reduction of testes, epididymis, and accessory sex organs weights and change in sperm characters; decease of sperm count and motility, and increase in sperm abnormalities. Liver functions tests such as Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were significantly increased. Moreover, administration of Ivermectin and/or Rafoxanide induced histopathological alterations in reproductive organs, liver and kidney tissues.

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1 - INTRODUCTION

Ivermectin, a broad –spectrum anti-parasitic agent mainly used in veterinary medicine, is active against numerous species of parasitic worms and arthropods (Del Giudice et al. 2003). Ivermectin is one the avermectins groups. The avermectins are a group of chemically related anthelmintics produced by fermentation of an actinomycete known as Streptomyces avermitilis, isolated from soil samples in Japan (Burg et al.,1979). Ivermectin is a mixture of avermectins containing more than 80% avermectin B1a and less than 20% avermectin B1b. These two components, B1a and B1b have very similar biological and toxicological properties (Lankas and Gordon, 1989).

Chhaiya et al (2012) reported that ivermectin has been used worldwide to treat patients with onchocerciasis and strongyloidiasis. It is also used against a wide range of endoparasites (nematodes) and ectoparasites (insects, acarine) of animals and humans.

Roy (2001) reported that, avermectins inhibit the motility and thereby causes paralysis of worms. Gamma-aminobutyric acid (GABA) release was increased from synaptosomes of the nervous system due to B1a component. Increased release of GABA cause hyperpolarization of post synaptic cell and inhibit contraction of muscle. Thus, worms are expelled in the similar fashion as Ascarids following piperazine therapy.

Laboratory tests showed that ingested avermectin B1a is absorbed into the blood stream by mammals and that it is eliminated from the body within 2 days via the feces. Also, when monkeys were given a single intravenous injection of avermectin B 1a, more than 90% of the dose was excreted in feces within 7 days of the dosing (Lankas and Gordon, 1989).

Pérez et al (2013) reported that ivermectin was effective against the equine intestinal Strongylus, taking a percentage of 76%.

Plumb and Pharm (2004) reported that Ivermectin has a long terminal half-life in most species. It is metabolized in the liver via oxidative pathways and is primarily excreted in the feces less than 5% of the drug (as parent compound or metabolites) excreted in the urine. Nourka and Agu (2001) reported that the oral Ivermectin was given in a single dose of 200mg/kg b.wt to 29 patients with scabies induced a93% resolution of pruritis with no side effects observed. The results showed that oral Ivermectin is a
promising effective 2nd safe alternative in both children and adults. Rafoxanide belongs to the group of halogenated salicylanilide anthelmintic agents used extensively for the control of liver flukes in sheep and cattle, and larvae of Oestrus ovis in sheep (Swan, 1998). Rafoxanide is active agent almost all mature and most immature(6 week old) Fasciola hepatica and Fasciola gigantica, adult Haemonchus species in cattle and sheep, and the parasitic larval stages of sheep nasal bot are susceptible (Swan and Mulders, 1993)

Swatipal et al. (2004) found that the Rafoxanide is slowly absorbed from the gastrointestinal tract, slowly eliminated; highly protein bound and persists for long time in blood of goats. Swan and Mulders (1993) reported that oral dosing Rafoxanide is absorbed from the small intestine into the blood stream peak plasma levels occurs between 24 and 48 hours.

Rafoxanide is extensively bound (>99%) to plasma proteins and has a long (16.6 days) terminal half and life. The blood is the primary source for the uptake of rafoxanide via the digestive system of the haematophagous helminthes (Benchou and Mckellar1993).

Swatipal et al. (2004) found that the clinical efficacy of Rafoxanide against fascioliasis was more if it was administered orally at 15 mg/kg instead of conventional dose of 7.5 mg/kg and at this dose level in goats did not exhibit any sign of toxicity.

2. Materials and Methods

2.1. Ivermectin

Ivermectin [Intermectin® 1% solution. The drug is produced by Horsterweg 26A5811 Ac castenrag

2.2. Rafoxanide

Rafoxanide : Flukanil® (Rafoxanide7.5% injection) is produced by pharmaswede. Egypt Company

2.3. Experimental design

Animals

The studies were carried out on 60 adult male albino rats of 130-170g body weight each and 140 days old. The animals were purchased from the Medical research Institute of Alexandria University. The study was conducted to evaluate the effect of Ivermectin or Rafoxanide and their coadministration in male rats; on fertility, liver, and kidney functions, 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 injection and distilled water at dose of 2ml/kg bwt orally

The second group: was injected with ivermectin (0.56mg/kg b.w. s.c.)

The third group: was given with rafoxanide (7.5mg/kg b.w. oral)

The fourth group: Rats were injected with ivermectin (0.56mg/kg b.w. s.c) + (rafoxanide 7.5mg/kg b.w oral). The dose of each drug was calculated according to Paget and Barnes (1964).

Treatment regimen was repeated to all groups after 30 days from the start of the experiment. Five rats from each treated and control group were killed after 2, 4, 8 weeks from the beginning of drug administration. Blood, body organs and epididymal contents were obtained from treated and control rats.

2.4. Blood sampling:

Two blood samples from each control and treated rats were taken from orbital plexus (inner canthus of the eye) under light ether anaesthesia using heparinized hematocrite tube. One sample was taken 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 sera. The sera were identified and stored in deep freezer at −20°C till used for biochemical analysis.

2.5. Fertility studies:

Rats were sacrificed by decapitation 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 sperm
were observed under high power lens power and the percentage of abnormal sperms was estimated and recorded.

b-Epididymal sperm count:
For counting epididymal sperms, a hemocytometer and a pipette were used. A drop of caudal 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.

2-6- Weight of internal body organs
After collection of the blood samples and epididymal sperm examination, testes, accessory sex organs [prostate and seminal vesicle] and epididymis were dissected out, grossly examined and weighed. The index weight [I.W] of each organ was calculated as described by Matousek (1969).

\[
\text{Index weight[I.W]} = \frac{\text{organ weight}}{\text{body weight}} \times 100
\]

2-7. Biochemical studies
Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were measured colourimetrically according to the method described by Reitman and Frankel (1957). The 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) and 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 by 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)

2.8. Hematological studies:

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

b-Pack cell volume (PCV) percent: Each blood sample was mixed then the microhaematocrit 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 pipeette. Double improved Neubauer haemocytometer was used in the count (Dacie and Lewis, 1984).

d-Total leukocytic count: Each blood sample was gently mixed then diluted by using Turk’s solution for total leukocytic count in respective blood count (Dacie and Lewis, 1984).

2.9. Histopathological 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 conventional 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 60 °C] 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) were examined.

2- 10- 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 administration of Rafoxanide (7.5 mg/kg b.w oral) or Ivermectin (0.56 mg/kg b.w.s.c) , and Ivermectin + rafoxanide, induced a significant decreased in index weight of testes after 4th week of the groups treated with Rafoxanide and interaction group. On the other hands, at 8th week of the experiment in all treated groups as compared with control group. Also, there was a significant reduction of weight of epididymis after 2nd week of the group treated with Rafoxanide as compared with control.
group and at 4\textsuperscript{th} 8\textsuperscript{th} week of the experiment in all treated groups. However, there was a significant reduction in weight of accessory sex glands after 2\textsuperscript{nd} week of the group treated with both ivermectin + rafoxanide and at 4\textsuperscript{th} 8\textsuperscript{th} week of the experiment in all treated groups. The degree of reduction in weight of accessory sex glands was more pronounced in rats treated with interaction group and Rafoxanide than other treatment (Table 1).

2-Effect on sperm count, motility and sperm abnormalities%:
There was a significant reduction in sperm cell count in all treated group after 4\textsuperscript{th} and 8\textsuperscript{th} weeks of the experiment as compared with control group. Also there was a significant reduction in the progressive sperm motility % after 2\textsuperscript{nd} week of Rafoxanide either alone or in combination with ivermectin and at 4\textsuperscript{th} and 8\textsuperscript{th} weeks in all treated groups of the experiment as compared with control group. However, there was a significant increase in the total sperm abnormality % in all treated groups at 4\textsuperscript{th} and 8\textsuperscript{th} week of the experiment as compared with control group. The degree of reduction in the sperm abnormality was more pronounced in rats treated with interaction group than other treatments (Table 2).

3-2-. Biochemical studies
The activities of ALT and ALP were significantly increased in all treated groups at the 2\textsuperscript{nd} 4\textsuperscript{th} and 8\textsuperscript{th} week of the experiment as compared with the control group. Also, a significant increase in the activity of AST in all treated groups at 4\textsuperscript{th} and 8\textsuperscript{th} weeks was seen (Table 3). However, there was a significant increase of total protein level of treated group, Ivermectin at 4\textsuperscript{th} weeks and did not produce any alteration in serum albumin level allover experimental periods as compared with control group. In addition, there was a significant increase of globulin level of treated group. Ivermectin at 4\textsuperscript{th} weeks and at 2\textsuperscript{nd} week of treated Rafoxanide group (Table 4). There were a significant increase in serum urea level in all treated group at the 2\textsuperscript{nd} 4\textsuperscript{th} and 8\textsuperscript{th} weeks of the experiment as compared with the control group. In addition, there was a significant increase in creatinine level at 2\textsuperscript{nd} 4\textsuperscript{th} week of ivermectin and interaction group and after 8\textsuperscript{th} weeks of group interaction group (Table 5).

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

There was a significant change in count of RBCs in all treated groups after the 8\textsuperscript{th} week of the experiment and at 2\textsuperscript{nd} and 4\textsuperscript{th} of Rafoxanide and interaction groups. Also, there was a significant change in the count WBCs of group treated with Rafoxanide-Ivermectin at 8\textsuperscript{th} weeks as compared with control group. There was a significant changes in the Hb after 4\textsuperscript{th} and 8\textsuperscript{th} week of the experiment in all treated groups as compared with control group. However, there was significant increase in PCV in all treated groups at the 4\textsuperscript{th} and 8\textsuperscript{th} week and at the 2\textsuperscript{nd} weeks of treated group rafoxanide and interaction group of the experiment as compared with control group (Table 6).

Table (1): The effect of administration of Ivermectin (0.56 mg/kg b.w.s.c ) or Rafoxanide (7.5 mg/kg b.w oral ) and their combined at interval of 30 days a part on the index weight of reproductive organs at different periods in adult male rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Testis index weight (g)</th>
<th>Epididymis index weight (g)</th>
<th>Accessory sex glands index weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Group</td>
<td>2\textsuperscript{nd} week</td>
<td>4\textsuperscript{th} week</td>
<td>8\textsuperscript{th} week</td>
</tr>
<tr>
<td>Control</td>
<td>1.20 ± 0.01A</td>
<td>1.16 ± 0.04A</td>
<td>1.18 ± 0.03A</td>
</tr>
<tr>
<td>Rafoxanide</td>
<td>1.17 ± 0.01A</td>
<td>1.11 ± 0.00A</td>
<td>1.10 ± 0.00B</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>1.17 ± 0.02A</td>
<td>1.12 ± 0.00A</td>
<td>1.11 ± 0.00B</td>
</tr>
<tr>
<td>Ivermectin+Rafoxanide</td>
<td>1.17 ± 0.01B</td>
<td>1.10 ± 0.01B</td>
<td>1.09 ± 0.01B</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error N=5. Means within the same column within each variable carrying different letters are significantly different (p<0.05)
Table (2): The effect of administration of Ivermectin (0.56 mg/kg b.w.s.c) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 30 days a part on fertility parameters at different periods in adult male rats

<table>
<thead>
<tr>
<th>Paramtrar</th>
<th>Sperm motility (%)</th>
<th>sperm count(×106/ml)</th>
<th>Sperm abnormalities(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time Group</strong></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week</td>
<td>4&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>8&lt;sup&gt;th&lt;/sup&gt; week</td>
</tr>
<tr>
<td>Control</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Rafoxanide</td>
<td>B</td>
<td>76.6±</td>
<td>85.3±</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>AB</td>
<td>B</td>
<td>75.6±</td>
</tr>
<tr>
<td>Ivermectin+ Rafoxanide</td>
<td>B</td>
<td>74.6±</td>
<td>72.6±</td>
</tr>
</tbody>
</table>

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

Table (3): The effect of administration of Ivermectin (0.56 mg/kg b.w.s.c) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 30 days a part on liver enzymes level at different periods in adult male rats

<table>
<thead>
<tr>
<th>Paramtrar</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time Group</strong></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week</td>
<td>4&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>8&lt;sup&gt;th&lt;/sup&gt; week</td>
</tr>
<tr>
<td>Control</td>
<td>B</td>
<td>29.25±</td>
<td>31.6±</td>
</tr>
<tr>
<td>Rafoxanide</td>
<td>A</td>
<td>31.0±</td>
<td>51.8±</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>A</td>
<td>32.2±</td>
<td>50.6±</td>
</tr>
<tr>
<td>Ivermectin+ Rafoxanide</td>
<td>A</td>
<td>32.6±</td>
<td>40.0±</td>
</tr>
</tbody>
</table>

Values within the same column within each variable carrying different letters are significantly different (p<0.05)

4. Histopathological examination

The microscopic examination of the livers in all treated groups revealed congestion of portal blood vessels and some lymphocytic aggregation. Also, severe congestion of the portal blood vessel of treated group of Rafoxanide at 8 weeks (Fig 1). The kidneys revealed, congestion of renal blood vessels and tubular dilation in all treated groups at 2<sup>nd</sup>, 4<sup>th</sup> and 8<sup>th</sup> weeks and after 4 weeks of treated group Ivermectin showed renal cortex with intertubular congestion, hemorrhages and lymphocytic infiltration (Fig 2). By testicular examination the seminiferous tubules separated by interstitial edematous fluids and spermatogenic cells inside their lumen in all treated groups at 4<sup>th</sup> and 8<sup>th</sup> weeks (Fig 3). The epididymis showed spermatozoal contents, edema and fibroplasia, interstitial lymphocytic infiltration aggregation in all treated groups at 2<sup>nd</sup>, 4<sup>th</sup> and 8<sup>th</sup> weeks. Moreover, dilation in treated group of Rafoxanide at 2<sup>nd</sup>, 4<sup>th</sup> and 8<sup>th</sup> weeks (Fig 4).
Table (4): The effect of administration of Ivermectin (0.56 mg/kg b.w.s.c) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 30 days on total protein, albumin and globulin level at different periods in adult male rats

<table>
<thead>
<tr>
<th>Paramtr</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week</td>
<td>4&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>8&lt;sup&gt;th&lt;/sup&gt; week</td>
</tr>
<tr>
<td>Control</td>
<td>5.56±0.31A</td>
<td>5.90±0.20B</td>
<td>5.96±0.39AB</td>
</tr>
<tr>
<td>Rafoxanide</td>
<td>5.80±0.20A</td>
<td>5.63±0.11B</td>
<td>5.24±0.10B</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>5.58±0.27A</td>
<td>6.46±0.22A</td>
<td>6.10±0.24A</td>
</tr>
<tr>
<td>Ivermectin+ Rafoxanide</td>
<td>5.66±0.12A</td>
<td>5.66±0.12B</td>
<td>5.8±0.12AB</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error N=5
Means within the same column within each variable carrying different letters are significantly different (p<0.05)

Table (5): The effect of administration of Ivermectin (0.56 mg/kg b.w.s.c) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 30 days on kidney function at different periods in adult male rats

<table>
<thead>
<tr>
<th>Paramtr</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Week</td>
<td>4&lt;sup&gt;th&lt;/sup&gt; Week</td>
</tr>
<tr>
<td>Control</td>
<td>22.68±0.72B</td>
<td>24.28±0.50C</td>
</tr>
<tr>
<td>Rafoxanide</td>
<td>33.04±0.67A</td>
<td>34.80±0.46B</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>33.02±0.36A</td>
<td>36.02±0.88AB</td>
</tr>
<tr>
<td>Ivermectin+ Rafoxanide</td>
<td>33.30±0.38A</td>
<td>37.50±0.74A</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error N=5
Means within the same column within each variable carrying different letters are significantly different (p<0.05)

Table (6): The effect of administration of Ivermectin (0.56 mg/kg b.w.s.c) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 30 days on hematological parameters count at different periods in adult male rats

<table>
<thead>
<tr>
<th>Paramtr</th>
<th>Pcv%</th>
<th>RBCs count (×106/cmm)</th>
<th>WBCs count (×103/cmm)</th>
<th>Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week</td>
<td>4&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>8&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week</td>
</tr>
<tr>
<td>Control</td>
<td>31.94±1.01A</td>
<td>32.70±1.80A</td>
<td>33.38±0.94A</td>
<td>3.77±0.37B</td>
</tr>
<tr>
<td>Rafoxanide</td>
<td>33.38±0.94B</td>
<td>38.74±3.06B</td>
<td>40.22±0.48A</td>
<td>5.77±0.46A</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>33.14±1.12AB</td>
<td>36.34±2.45B</td>
<td>38.90±3.16B</td>
<td>4.68±0.62AB</td>
</tr>
<tr>
<td>Ivermectin + Rafoxanide</td>
<td>35.52±0.65B</td>
<td>39.22±2.18B</td>
<td>42.13±1.26B</td>
<td>5.37±0.48A</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error N=5
Means within the same column within each variable carrying different letters are significantly different (p<0.05)
The examination of prostate gland and its secretory epithelial cells, luminal cellular debris, congestion and edema in all treated groups at 2nd, 4th and 8th weeks were recorded. The degree was more prominent at 4th and 8th week in the group interaction (Ivermectin +Rafoxanide) (Fig. 5). Regards to seminal vesicles, secretions, congestion and edema were seen in all treated groups was recorded .Also, there was severe congestion of the interstitial blood vessels in treated interaction group at 4th weeks (Fig 6).

1. DISCUSSION
Ivermectin together with antitrematodal drugs may be seen in the same prescription, therefore, the present study was carried out to investigate the effect of ivermectin and/or its interaction with rafoxanide on male fertility, blood picture, liver and kidney functions in rats. Animal were treated with two doses separated by 4 weeks from either ivermectin (0.56 mg/kg b.wt.s.c.) and/or rafoxanide (7.5 mg/kg b.wt orally).
The duration of the present study lasts for 2 months to cover the spermatogenic cycle in rats which ranges from 48-56 days (Clermont and Harvey, 1965). The present results are compatible with those reported by Zaied (1995 & 2004) and Wains (1996) they found that injection of ivermectin (0.3 mg/kg b.wt, s.c) caused a significant decrease in testes, epididymis and accessory sex organs index weights of rabbits and rats.

Concerning the effect of ivermectin and/or rafoxanide on the epididymal sperm characters, the obtained results revealed that, there was a significant reduction in sperm cell concentration in all treated groups at 4th and 8th weeks of the experiment. Also, there was a significant increase in the progressive sperm motility percent in all 4th and 8th weeks of the experiment and after 2nd week of treatment with Rafoxanide and interaction group. Moreover, there was a significant increase in total sperm abnormalities percent in all treated groups at 4th and 8th weeks of the experiment. The degree of increase in the total sperm abnormalities %, was more pronounced in rats treated interaction groups.

These findings could be attributed to the obtained histopathological changes in testes and epididymis in the present study which were in from of congestion of the interstitial blood vessels of the seminiferous tubules with less active spermatogenesis and excess of immature exfoliated spermatogenic cells, with the luminal epididymal contents of spermatozoa. Ivermectin + Rafoxanide these lesions were increased in the severity in interaction group, where there was spermatocytic exfoliation, spermatozoa contents and edema and fibroplasias. histopathological findings in accessory sex organs represented as lack of the acinars secretion in addition to fibroplasias and mild epithelial hyperplasia in seminal vesicles in all treated groups.

These results are in agreement with those obtained by El-shaieb and Mahmoud (2000), who found that injection of mature Balady male rabbits with ivermectin resulted in arrested spermatogenesis, some seminiferous tubules were widely dilated and lined with one layer of spermatogenic germ cells. The lumina of some tubules contained desquamated and necrotic germ cells. Multinucleated giant cells could be seen. The interstitial tissue was thick, the epididymis was free from sperm. Also, Zaied (2004) reported that injection of male rats with single therapeutic dose of ivermectin (0.3 mg/kg b.wt.) induced a significant decrease in epididymal and testicular sperm count and progressive sperm motility. The testes showed interstitial oedema, giant cell formation with necrosis of spermatogenic cell in association with congestion and oedema in epididymis, prostate and seminal vesicles.

In the current study, it was found that administration of ivermectin (0.56 mg/kg b.wt. s.c) and/or rafoxanide (7.5 mg/kg b.wt. oral) injected twice separated with 4 weeks in mature male rats induced significant changes in RBCs in count in all treated
groups after the 8th week of the experiment and at 2nd and 4th week in Rafoxanide and interaction groups. Also, there was a significant change in the count of WBCs of interaction group at 8th week as compared with control group. There was a significant change in the Hb after 4th and 8th weeks of the experiment in all treated groups as compared with control group. However, there was a significant increase in PCV in all treated groups at the 4th and 8th week and at the 2nd week of treated group rafoxanide and interaction group of the experiment as compared with control group. The obtained results disagree with those recorded by Morsy (2009) who stated that administration of rafoxanide induced insignificant decrease in erythrocytic count haemoglobin content and packed cell volume. Our results are in agreement with those recorded by Zaied (2004) who demonstrated that injection of male rats with ivermectin at a dose of (0.3mg/kg b.wt.s.c) resulted in, insignificant changes in Hb concentration, Pcv% , RBCs and WBCs counts.

The biochemical studies were carried out to investigate the degree of hepatic and/or renal damage following the parenteral administration of the drugs. The activities of ALT and ALP were significantly increased in all treated groups at 2nd, 4th and 8th week. Also, significantly increased of activity of AST in all treated group at 4th and 8th weeks of the experiment as compared with the control group. These results were supported by the findings reported by Morsy (2009) who mentioned that administration of rafoxanide induce significant elevation in AST, ALT and ALP. El-Shaieb and Mohamed (2000) who recorded that injection of mature male rabbits with 3 double therapeutic doses (0.4mg/kg b.wt.) of ivermectin with (1 week interval) resulted in elevation in serum AST, ALT and ALP activities. Microscopically, the hepatic parenchyma was congested and showing hydropic degeneration, focal coagulative necrosis and the portal areas were over distended with connective tissue proliferation infiltrated with round cells. Also, Zaied (2004) reported that injection of male rats with ivermectin at a dose of (0.3mg/kg b.wt.s.c) induced significant increase in serum AST, ALT and ALP activities. Congestion with lymphocytic aggregation particularly the portal areas and mild degrees of hepatocytic degeneration were found. The present work demonstrated that injection of ivermectin and rafoxanide induced nonsignificant change in albumin levels in all treated groups. However, there was a significant increase of total protein level of treated group Ivermectin at 4th weeks and there was a significant increase of globulin level of treated group Ivermectin at 4th weeks and at 2nd week of treated Rafoxanide group.

The results are in agreement with Zaied (2004) who recorded that administration of ivermectin (0.3 mg/kg b. wt. s. c) of male rats induced insignificant changes in serum total protein albumin and globulin levels. On the other hand, The results disagree with those reported that by Emam and AbdAlla (2000) who noted that a significant reduction in serum total protein level in rabbits treated with ivermectin was recorded.

The obtained results showed that administration of ivermectin and rafoxanide, there were significantly increased serum levels of urea level in all treated groups at the 2nd, 4th and 8th week of the experiment as compared with control group. Also, there was a significant increased creatinine level at 2nd and 4th week of ivermectin and interaction group and after 8th weeks of group interaction group. The results are agree with those reported by Zaied (2004) who found that injection of ivermectin (0.3mg/kg b.wt.s.c) in male rats, induced a significant increase in serum levels of urea and creatinine.

CONCLUSION

It could be concluded that administration of Ivermectin and/or Rafoxanide 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 Ivermectin and Rafoxanide carefully to avoid possible adverse effects especially during breeding season.

REFERENCES


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