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Some Pharmacodynamics Effects of Rafoxanide and Its Interaction With Fenbendazole

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ABSTRACT

This study was carried-out to study the pharmacodynamics characters of rafoxanide with fenbendazole and the interaction between them through studing its effects on, fertility (reproductive organs weights, progressive motility, epididmal sperm count and sperm and abnormalities), Moreover, its effect on some hematological parameters including (WBCs count, differential leucocytic count, RBCs count, and Hb %, platelets count and PCV %), also, the effects on some biochemical parameters including (ALT, AST, and Albumin, total protein, globulin, urea and creatinine) were determined. Sixty mature Albino rats weighing from 140 – 180 gm each of 4 – 5 month old were used in this study. The animals were divided into 4 equal groups each of 15 rats, the first group: was left without treatment and kept as a control group, the second group: Given orally Fenbendazole orally at dose level (37.5 mg/Kg), third group: Given orally Rafoxanide at a dose (7.5 mg/Kg) orally) and the fourth group: Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) orally).

Our results concluded that, The using of Fenbendazole for treatment of helminthes parasites improved the index weight of accessary sex glands, tests index weight and epidedymis index weight. Also, increased the values of WBCs, count, Hb, PCV, serum protein, albumin globulin, serum urea, serum creatinine level observed in Fenbendazole treated group compared with rafoxanide treated group or its combination. But it decreased the level of sperm motility, sperm count and in Fenbendazole and the higher sperm abnormalities level observed in the group treated with Fenbendazole. While, the rafoxanide improved the level of sperm motality, count and decrese percentage of abnormalities than Fenbendazole or its combination. The histopathological results cleared that, there is a severe histological changes in the group treated with Fenbendazole and Rafoxanide than the control group and their combination.

1. INTRODUCTION

Helminth infections caused by roundworms (nematodes) and flatworms (platyhelminths) comprise the greatest group of the neglected tropical diseases (NTDs) (Hotez et al., 2006). An estimated 11.5 million disability adjusted life years (DALYs) are attributed to intestinal nematode infections, schistosomiasis, lymphatic filariasis, onchocerciasis, food-borne trematodiasis, cysticercosis and echinococcosis (Hotez et al., 2006 and Murray et al., 2012). Preventive chemotherapy is the strategy of choice to control schistosomiasis, soil-transmitted helminthiases, lymphatic filariasis, onchocerciasis and food-borne trematodiases , limited tools are available to treat these infections. Yet clear targets have been set to eliminate and control several of these diseases. (Pedrique et al., 2013 and Andrews et al., 2014).

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 (Mossallam et al., 2007 and

EMA, 2014). 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 Used as a fasciolicide in cattle and sheep.Rafoxanide is also active against gastrointestinal nematodes (Haemonchus, Bunostomum, Oesophagostomum, and Gaigeria species) and against the sheep nasal bot fly (Oestrus ovis). Rafoxanide products are currently marketed in the European union (EU) for the treatment of cattle and sheep (EMA, 2014 and Prchal et al., 2016) Fenbendazole is a benzimidazole anthelmintic that is metabolised in mammals to a series of other benzimidazoles including oxfendazole. It is used for the control of gastrointestinal roundworms, lung worms and tape worms. (Huerkamp et al., 2000). Fenbendazole (FBZ) is a broad-spectrum benzimidazole anthelmintic drug that has gained widespread use for the treatment and prophylaxis of pinworm infection (Pritchett and Jounston et al., 2002 and Villar et al., 2007). Fenbendazole has been approved by the European Agency for the Evaluation of Medical Products (EMEA) for the use as an anthelmintic in swine. It is administered with feed in the dose of 3-5 mg/kg for 5-10 d, to control Ascaris, Trichuris, Oesophagostomum, Strongyloides and Hyostrongylus spp. (Crescenzo et al., 1994).

Rafoxinaide combinations with other anthelminitics can improved its action and efficacy in treating the gastro-intestinal parasites. Lanusse and Prichard, 1993; Knox et al., 1994 and Szprengier-Juszkiewicz et al., 1998). Also, El-Banna et al. (2008) revealed that the combination of Rafoxanide with Ivermectin (IVM) in sheep and calves increased the absorption of IVM and delayed its elimination.. (Cringoli et al. 2006 and Ram et al. 2007)

This study was carried-out to study the pharmacodynamics characters of Rafoxanide with Fenbendazole and the interaction between them through studing their effects on, fertility (reproductive organs weights, progressive motility, epididmal sperm count and sperm and abnormalities), Moreover, its effect on some hematological parameters including (WBCs count, differential leucocytic count, RBCs count, and Hb %, platelets count and PCV %), also, the effects on some biochemical parameters including (ALT, AST, and Albumin, total protein, globulin, urea and creatinine) were determined Also their histopathological effects on liver, kidney, testes epididymis and accessory gland.

2. MATERIALS AND METHODS

2.1. Drug.

a-Rafoxanide: Rafinide® suspension 2.5 %. Aveco – Jordan.

b-Fenbendazole (FBZ): Curosol® 10 % -Oral drensh. Produced by Univet , Ireland .

2.2.Experimental design:

a.Animals:The present work is a trial to study oral administration effects of Rafoxanide , fenbendazole and the interaction between them on the reproductive system in male Albino rats. Moreover, some haematological, biochemical, and histopathological changes were also examined.

b.Animal grouping:Sixty mature Albino rats weighing from 140 – 180 gm each of 4 – 5 month old age were used in this study.

c. The animals were divided into 4 equal groups each of 15 rats.

The first group**:** Was kept as a control group.

The second group: Was given orally Fenbendazole orally at dose level (37.5 mg/Kg).

Third group: Was given orally Rafoxanide at a dose (7.5 mg/Kg) orally).

Fourth group: Fenbendazole (37.5 mg/Kg) + Rafoxanide

(7.5 mg/Kg) orally)

The dose was calculated according to (Paget and Burns, 1964).

2.3-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 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 sera. The sera were separated and stored in deep freezer at −20ºC till being used for biochemical analysis.

2.4-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 epididymal content 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 epididymal content and mixed well then the film fixed by gentle heating was spread on the slide. Three hundred sperm were examined for estimating abnormalities .

b. Epididymal sperm count**:**

For counting epidiymal sperms, a hemocytometer and a red 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 for 1-2 mintes 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 and fill haemocytometer by the capillary action then count the sperm heads in the counting chamber.

2.5-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). Index weight[I.W] = (organ weight/ body weight) X 100

2.6. 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 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):

Hb was determined according to the method described by Benjamin (1978) by using Sahlis haemocytometer .

b-Packed 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 gently mixed then diluted 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, accossary 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 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.

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

3. RESULTS

3. 1.Effects of Fenbendazole and Rafoxanide and their combination on testes index weight:

Our results that observed in Table (1) cleared that, there is a significant differences of the testes index weight, epidedymis index weight and accessory glands index weight weight among different treatment groups and at different period of experiment ($P < 0.01$).The higher index weight observed in the Fenbendazole (37.5 mg/Kg) as the higher level of this group was observed after 8th week of experiment, followed by the group treated with Rafoxanide (7.5 mg/Kg) orally, as its higher level was observed after 4th weeks. Followed by the groups treated with , the combination with of Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) orally, and the higher heart index weight of this group observed after 4th weeks of experiments.

While, the lower index weight observed in control group and the higher level in this group was observed after 2nd weeks of experiment.

3.2. Effects of Fenbendazole and Rafoxanide and their combination on sperm charactersc (motility, count and abnormalities) cleared that, there is a significant differences of the sperm motility, sperm count percent among different treatment groups and at different periods of experiment ($P < 0.01$).

The good sperm characters observed in Fenbendazole (37.5 mg/Kg) as the higher level of this group observed after 2nd week of experiment. Followed by control group as its higher level in this group was observed after 2nd week of experiment and Rafoxanide (7.5 mg/Kg) orally, as its higher level observed after 2nd week.The lower level pf sperm characters observed in the group treated with , the combination of Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) orally, and the higher accessory gland index weight was observed after 4th week of experiments.

3.3.Effects of Fenbendazole and Rafoxanide and their combination on serum enzymes (ALT, AST and ALP level):

Our results that observed in Table (2) cleared that, there is a significant differences of the ALT levels among different treatment groups and at different period of experiment ($P < 0.01$).

The higher serum enzymes (ALT, AST and ALP): level was observed in Rafoxanide (7.5 mg/Kg) orally treated group, as its higher level observed after 4th weeks of experiment. Followed by control group as its higher ;evel in this group observed after the 2nd week of experiment. Followed by the combination of Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) orally, and the higher ALT level was observed after the 4th week of experiments. The lower (ALT, AST and ALP) level observed in Fenbendazole (37.5 mg/Kg) treated group as the higher level of this group observed the 4th week of experiment.

3.4.Effects of Fenbendazole and Rafoxanide and their combination on serum albumin level (albumin, globulin and total serum proteins):

Our results that presented in Table (3) cleared that, there is a significant differences of the albumin levels among different treatment groups and at different period of experiment ($P < 0.01$). The higher serum proteins level (albumin, globulin and serum proteins) was observed in the group treated with Fenbendazole (37.5 mg/Kg) and the higher level of this group was observed after the 2nd week of experiment. Followed by the Rafoxanide (7.5 mg/Kg) orally treated group, as its higher was level observed after the 8th week of experiment. Followed by control group as its higher level was observed after 4th week of experiment. The lower serum protein level was observed in the group treated with the combination of Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) treated groups and the higher level of this group was observed after 8th weeks of experiments.

3.5.Effects of Fenbendazole and Rafoxanide and their combination on serum urea level: Our results persented in Table (4) cleared that, there is significant differences of serum urea levels among different treatment groups and at different periods of experiment ($P < 0.01$). The higher serum urea and creatinine levels was observed in the group treated with Fenbendazole (37.5 mg/Kg) treated group as the higher level of this group was observed the after 4th week of experiment. While, the serum urea and creatinine level in control group reached its highest level at 8th week of experiment.

While, the serum urea and creatinine in the group treated with combination of Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) treated groups and the higher level of this group observed after 4th weeks of experiments. The lower serum urea level was observed in the group treated with Rafoxanide (7.5 mg/Kg) while orally, with its higher level observed after the 4th week of experiment.

3.6. Hematological studies Red blood corpuscles (RBCs) ,white blood cells (WBCs) haemoglobin (Hb) Concentration (g/dl) and packed cell volume percent (PCV%)}.Effects of Fenbendazole and Rafoxanide and their combination on PCV, RBCs, count Hb and WBCs count level:

Our results presented in Table (12) and Fig (12) cleared that, there is a significant differences of the RBCs count among different treatment groups and at different period of experiment ($P < 0.01$). The higher PCV%, RBCs, count Hb g% and WBCs values observed in Rafoxanide (7.5 mg/Kg) orally, as its higher level was observed the after 8th weeks of experiment. Followed by Fenbendazole (37.5 mg/Kg) as the higher level of this group was observed after the 4th week of experiment. The valuos of PCV, RBCs, Hb and WBCs in control group showed a higher value after 8th weeks of experiment. The lower PCV, RBCs, Hb and WBCs valous observed in the combination with of Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) orally, and the higher RBCs count observed after 1st weeks of experiments.

Table (1): The effect of administration of Fenbendazole (37.5 mg/Kg) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 14 days a part on the index weight of reproductive organs at different periods in adult male rats.

Parameter	**Tests index weight**			**epidedymis index weight**			Accessory glands index weight		
Time Group	**2nd weeks**	**4th weeks**	**8th weeks**	**2nd weeks**	**4th weeks**	**8th weeks**	**2nd weeks**	**4th weeks**	**8th weeks**
Control	Aa 1.07± 0.05	Cb 0.96± 0.22	Ba 1.07± 0.04	Ca 0.58± 0.02	Aa 0.56± 0.09	Cb 0.41± 0.03	Bb 0.66± 0.03	Bc 0.53± 0.04	Aa 0.74± 0.06
Fenbendazole	Ac 1.01± 0.03	Bb 1.19± 0.04	Aa 1.28± 0.04	Aa 0.70± 0.02	Bb 0.43± 0.02	Cc 0.38± .02	Aa 0.73± 0.01	Aa 0.73± 0.04	Bb 0.59± 0.04
Rafoxanide	Ab 1.06± 0.03	Aa 1.23± 0.07	Aa 1.22± 0.02	Ba 0.68± 0.03	Cc 0.38± 0.03	Bb 0.46± 0.03	Aa 0.78± 0.02	Bb 0.56± 0.08	Bb 0.57± 0.08
Fenbendazole + Rafoxanide	Aa 1.05± 0.05	Ca 1.09± 0.08	Ba 1.04± 0.04	Db 0.38± 0.03	Cb 0.36± 0.07	Aa 0.53± 0.04	Cc 0.42± 0.04	Cb 0.49± 0.09	Aa 0.70± 0.04

Capital letters : Means within the same column of different letters are significantly different ($p<0.05$).
Small letters : Means within the same raw of different letters are significantly different ($p<0.05$).

Table (2): The effect of administration of Fenbendazole (37.5 mg/Kg) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 14 days a part on on fertility parameters at different periods in adult male rats.

Parameter	**Sperm motility (%)**			**sperm count(×106/ml)**			**Sperm abnormalities(%)**		
Time Group	**2nd weeks**	**4th weeks**	**8th weeks**	**2nd weeks**	**4th weeks**	**8th weeks**	**2nd weeks**	**4th weeks**	**8th weeks**
Control	Aa 92.00± 2.00	Bb 84.00± 1.87	Bb 81.00± 2.45	Aa 329.00± 7.31	Bc 241.00± 20.70	Bb 247.00± 6.63	Bb 4.40± 0.24	Ca 12.60± 2.89	Ca 12.80± 2.40
Fenbendazole	Aa 93.80± 1.71	Cc 81.00± 3.32	Ab 88.00± 1.22	Ba 313.00± 3.74	Dc 227.00± 8.89	Cb 241.00± 8.86	Bb 4.40± 0.98	Db 4.00± 0.95	Aa 28.80± 5.66
Rafoxanide	Aa 92.00± 1.22	Dc 75.00± 2.74	Ab 86.00± 1.87	Ca 305.00± 5.92	Cc 233.00± 16.78	Ab 267.00± 13.93	Bc 3.00± 0.32	Ab 24.60± 3.72	Aa 28.20± 5.07
Fenbendazole + Rafoxanide	Bb 72.00± 1.22	Aa 88.00± 1.22	Aa 87.00± 2.00	Db 234.00± 17.56	Aa 263.00± 10.68	Dc 180.00± 24.14	Ac 9.00± 1.41	Ba 29.40± 7.96	Bb 22.20± 6.04

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Table (3): The effect of administration of Fenbendazole (37.5 mg/Kg) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 14 days a part on liver enzymes level at different periods in adult male rats

Parameter	**ALT (U/L)**			**AST (U/L)**			**ALP (U/L)**		
Time	**2nd**	**4th**	**8th**	**2nd**	**4th**	**8th**	**2nd weeks**	**4th**	**8th**
Group	**weeks**	**weeks**	**weeks**	**weeks**	**weeks**	**weeks**		**weeks**	**weeks**
Control	Aa 66.00± 4.18	Bb 56.80± 3.29	Cc 42.40± 3.23	Ab 187.00± 3.08	Cc 163.20± 5.94	Ca 192.80± 14.62	Ba 254.40± 11.89	Bb 248.60± 19.48	Dc 189.30± 43.53
Fenbendazole	Da 58.80± 2.06	Dc 49.00± 2.47	Bb 51.00± 4.30	Cc 181.20± 7.60	Aa 235.20± 12.71	Cb 192.60± 13.85	Cb 252.40± 11.86	Dc 208.74± 9.75	Aa 287.60± 27.03
Rafoxanide	Cc 62.80± 3.62	Aa 75.80± 4.89	Ab 63.00± 4.14	Bb 184.60± 5.46	Dc 159.40± 9.74	Ba 243.40± 34.05	Dc 246.00± 6.87	Aa 353.00± 32.46	Bb 257.00± 17.43
Fenbendazole + Rafoxanide	Ba 64.60± 5.70	Cb 54.80± 3.57	Bc 50.80± 4.98	Dc 165.80± 13.60	Bb 190.80± 16.62	Aa 328.20± 30.57	Aa 363.60± 39.02	Cb 238.00± 25.99	Cc 197.80± 17.80

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Table (4). The effect of administration of Fenbendazole (37.5 mg/Kg) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 14 days a part on liver enzymes level at different periods in adult male rats.

Parameter	Total protein (g/dl)			Albumin (g/dl)			Globulin (g/dl)		
Time	**2nd weeks**	**4th**	**8th**	**2nd weeks**	**4th**	**8th**	**2nd weeks**	**4th**	**8th**
Group		**weeks**	**weeks**		**weeks**	**weeks**		**weeks**	**weeks**
Control	Cc 6.79±0.28	Aa 7.32±0.41	Ab 7.10±0.20	Aa 4.00±0.09	Ab 3.97±0.06	Ab 3.81±0.06	Cb 2.79±0.26	Aa 3.35±0.43	Aa 3.28±0.22
Fenbendazole	A a 7.86±1.10	Bb 6.88±0.23	Bc 6.26±0.20	Aa 4.16±0.10	Ab 3.79±0.08	Ab 3.76±0.09	Aa 3.70±1.12	Bb 3.09±0.17	Bc 2.50±0.20
Rafoxanide	Bc 7.08±0.10	Ab 7.15±0.45	Aa 7.37±0.28	Ba 3.92±0.09	Aa 3.86±0.07	Aa 3.94±0.11	Bc 3.16±0.08	Ab 3.29±0.44	Aa 3.43±0.22
Fenbendazole + Rafoxanide	Ba 7.13±0.32	Cb 6.27±0.19	Aa 7.16±0.14	Aa 4.08±0.06	Bc 3.46±0.15	Ab 3.90±0.08	Bb 3.05±0.34	Bc 2.81±0.13	Aa 3.26±0.13

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Table (5) . The effect of administration of Fenbendazole (37.5 mg/Kg) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 14 days a part on kidney function at different periods in adult male rats

Parameter	Creatinine (mg/dl)			Urea (mg/dl)		
Time	**2nd**	**4th**	**8th**	**2nd weeks**	**4th**	**8th**
Group	**weeks**	**weeks**	**weeks**		**weeks**	**weeks**
Control	Bb 0.55±0.01	Bb 0.53±0.01	Aa 0.85±0.02	Bb 23.46±1.04	Db 22.64±0.50	Ba 30.52±1.98
Fenbendazole	Bb 0.54±0.01	Aa 0.88±0.05	Cb 0.54±0.02	Bc 23.26±0.87	Aa 34.02±0.56	BCb 28.50±1.84
Rafoxanide	Aa 0.81± 0.02	Bb 0.53± 0.02	Cb 0.51± 0.03	Bc 24.80± 0.97	Ba 29.57± 1.31	Cb 26.22± 1.92
Fenbendazole + Rafoxanide	Bb 0.53± 0.03	Bb 0.54± 0.01	Ba 0.77± 0.04	Aa 30.66± 0.78	Cb 25.56± 3.42	Aa 32.30± 1.59

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Table (6) . The effect of administration of Fenbendazole (37.5 mg/Kg) or Rafoxanide (7.5 mg/kg b.w oral) and their combined at interval of 14 days a part on hematological parameters count at different periods in adult male rats

Parameter	PCV%			RBCs count (×106/cmm)			WBCs count (×103/cmm			Hb (g/dl)		
Time	2nd	4th	8th	2nd	4th	8th	2nd	4th	8th	2nd	4th	8th
Group	weeks	weeks	weeks	weeks	weeks	weeks	weeks	weeks	weeks	weeks	weeks	weeks
Control	Aa 39.86±0.88	Cc 35.12±0.72	Bb 38.48±0.67	Aa 7.14±0.23	Bb 6.80±0.06	Aa 7.24±0.05	Cb 6.54±0.45	Ca 7.26±0.43	Ba 7.36±1.28	Aa 14.00±0.25	Bb 13.32±0.12	Aa 14.40±0.21
Fenbendazole	Cb 36.40±0.43	Bb 36.68±0.63	Aa 41.66±0.56	Aa 7.16±0.12	Aa 7.31±0.10	Aa 7.25±0.17	Bc 7.36±0.41	Aa 11.14±0.51	Ab 8.52±0.60	Bb 13.80±0.22	Aa 14.16±0.14	Aa 14.56±0.23
Rafoxanide	Bb 38.68±0.96	Aa 41.36±0.40	Cc 37.98±0.74	Aa 7.09±0.17	Aa 7.17±0.06	Aa 7.56±0.15	Cc 6.04±0.35	Ba 10.38±0.54	Ab 8.74±0.53	Aa 14.12±0.36	Bb 13.90±0.64	Aa 14.22±0.39
Fenbendazole + Rafoxanide	Aa 39.02±0.91	Dc 33.18±0.46	Db 35.52±1.28	Aa 7.05±0.14	Bb 6.66±0.13	Bb 6.91±0.27	Aa 11.22±1.33	Bb 10.70±1.67	Bc 7.02±0.08	Ba 13.88±0.34	Cb 12.88±0.09	Ba 13.56±0.37

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3.7. Histopathological results:

Microscopic findings:

The most important histopathological features that appeared due to Some pharmacodynamics effect of Rafoxanide and its interaction with fenbendazole includes testicular lobules showing normal spermatogensis and germinal epithelium (Fig, 1), epididymis showing normal semen in lumen (Fig, 2), liver showing normal hepatic vein (Fig, 3), there is a marked congetion of testicular blood vessel(arrow) (Fig, 4), marked congetion of testicular blood vessel (Fig, 5), kidney showing some degenerated glomerulus) (Fig, 6), marked congetion of testicular blood vessel (Fig, 7) and congetion of hepatic blood vessel (Fig (8).

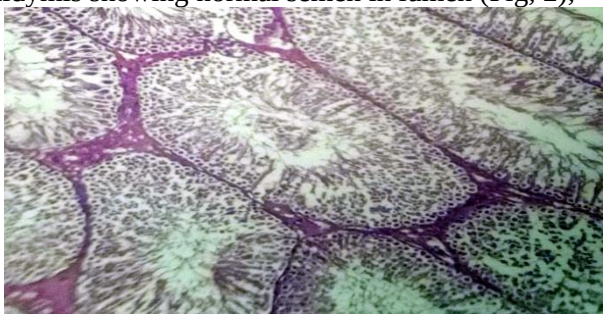

Fig (1): gpc: testis of mature male rat of control group showing normal spermatogensis and germinal epithelium(h&e,x250)

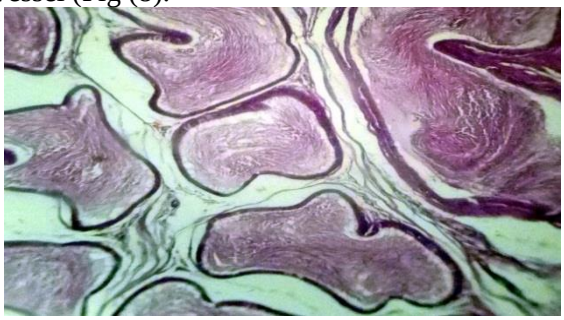

Fig (2): gpc: Epididymis of ,ature male rat of controul group showing normal semen in lumen(h&e,x250)

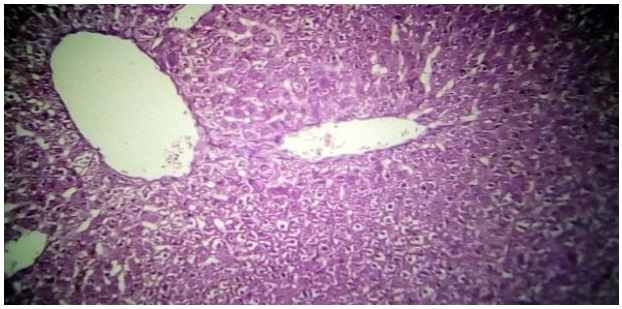

Fig (3): gp1:liver of mature male rat, 2 weeks post fenbendazole (37.5 mg / kg, b,wt.orally) adminstration showing normal hepatic vein(h&e,x100)

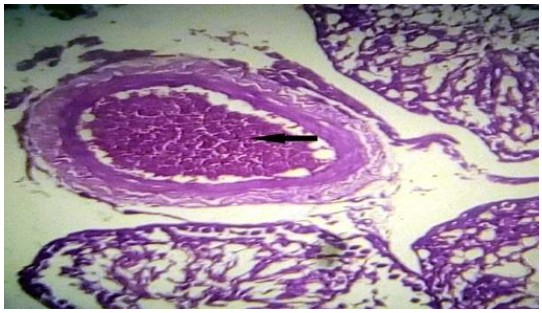

Fig (4): Gp1: Testis of mature male rat, 2weeks post fenbendazole (37.5 mg / kg, b,wt.orally) adminstration showing, markedcongetion of testicular blood vessel(arrow)(h&e,x250)

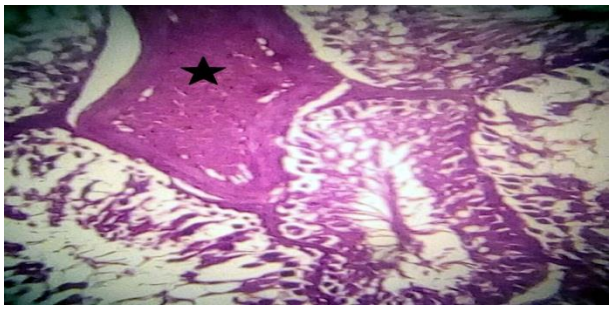

Fig (5): Gp1: Testis of mature male rat, 2weeks post fenbendazole (37.5 mg / kg, b,wt.orally) adminstration showing, marked congetion of testicular blood vessel(star)(h&e,x250gp1:some

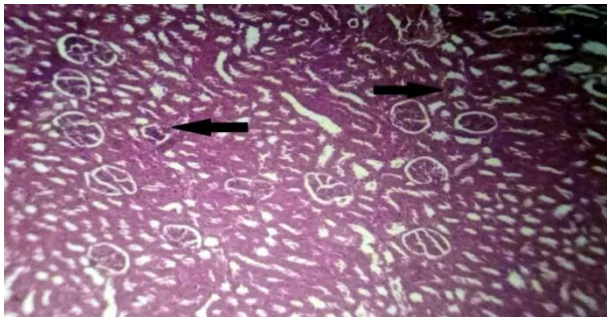

Fig (6): gp2: kidney of mature male rat, 4 weeks post fenbendazole showing some degenerated glomerulus (arrow)(h&e,x100)

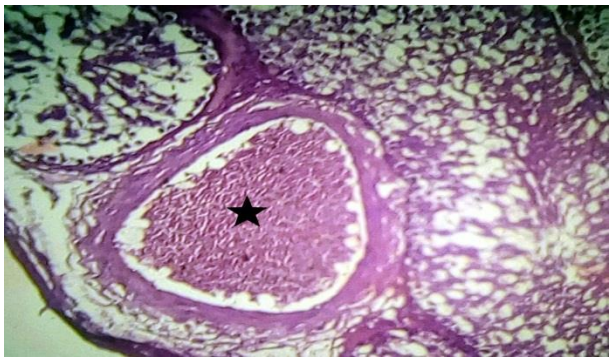

Fig (7): Gp3: Testis of mature male rat, 8weeks post fenbendazole (37.5 mg / kg, b,wt.orally) adminstration showing, marked congetion of testicular blood vessel(h&e,x250)

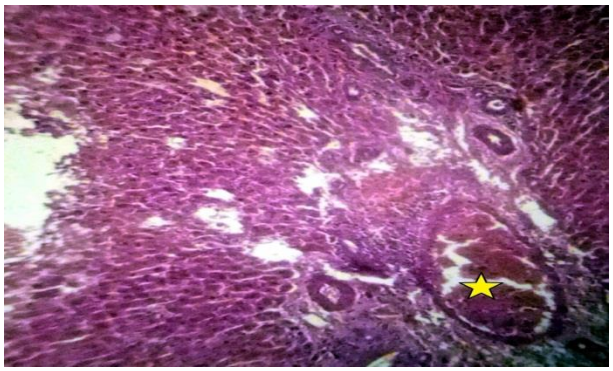

Fig (8): Gp4: Liver of mature male rat, 2weeks post Rafoxanide (7.5 mg/kg,b,wt.orally) administration showing congetion of hepatic blood vessel(star)(h&e,x250)

4-DISCUSSION

Our results revealed that the effect of Fenbendazole and Rafoxanide and their combination on accessory gland index weight_ showed its hights valous in the groups treated with Rafoxanide (7.5 mg/Kg) orally) after 4th week, Followed by Fenbendazole (37.5 mg/Kg) after 8 weeks of experiments. The reported findings agreed with those recorded by Dixit et al. (2018) who reported that administration of albendazole induced significant decrease in the weights of accessory glands. While, the effects of Fenbendazole and Rafoxanide and their combination on WBCs level cleared that, the higher WBCs was level observed in Fenbendazole (37.5 mg/Kg) after the 4th week of experiment. While, our results about the effects of Fenbendazole and Rafoxanide and their combination on WBCs level cleared that, the highest WBCs level was concerning observed in Fenbendazole (37.5 mg/Kg) after 4th weeks of experiment. Meanwhile, our results was the effects of Fenbendazole and Rafoxanide and their combination on Hb level cleared that, the highest Hb level was observed in Fenbendazole (37.5 mg/Kg) after 8th weeks of experiment. Meanwhile, our results about the effects of Fenbendazole and Rafoxanide and their combination on RBCs level, the higher RBCs level observed in Rafoxanide (7.5 mg/Kg) orally, after 8th weeks of experiment.. Meanwhile, our results on the effects of Fenbendazole and Rafoxanide and their combination on PCV level, the highest PCV value was observed in Fenbendazole (37.5 mg/Kg) after 8th weeks of experiment. The previously mentioned findings could be attributed to the Fenbendazole + Rafoxanide decrease the activity and livability of internal parasites that it will improve the health condition of the animal with improvement of feed utilization with improvemet of Hb concentration and this results agree with those of (Sibgh et al., 2017), where they reported, treatment of internal and external parasites will improved the health condition of the animals with improvement of Hb %.

The results of fenbendazole attributed to the Fenbendazole induced elimination of parasites and improved the health condition of the liver these results agreed with those of, (Singh et al. 2017) where they reported that, ricobendazole or fenbendazole administration induced the elimination of flukes and healing of the majority of hepatic lesions but did not prevent severe hepatic damage produced by later infections. This will improve the body conditions of the cattle and animals and improve the RBCs and WBCs level.

Meanwhile, the effects of Fenbendazole and Rafoxanide and their combination on ALT , AST, alkaline phosphatase level cleared that, the higher ALT and AST level observed in Rafoxanide (7.5 mg/Kg) after 4th weeks of experiment. Meanwhile, the lower ALT was observed in Fenbendazole (37.5 mg/Kg) treated group after the 4th week of experiment.

The histopathological results supported this findings, in Fenbendazole showed that, the livers of the treated rats showed congestion and dilatation of portal veins. Multifocally, there were small areas of hepatocytes necrosis characterized by loss of architecture and replaced by aggregates of mononuclear inflammatory cells. Occasionally, the necrotic hepatocytes were replaced by structureless eosinophilic substance infiltrated by lymphocytes, while the surrounding hepatocytes exhibited mild fatty change enlarged by a discrete clear cytoplasmic vacuole. (Malomo et al., 1995).

The Alanine aminotransferase activity in the blood are increased in conditions in which cells are damaged or dead (Jimoh and Odutuga, 2001). The significant reduction in the liver and kidney ALT and AST activities in all the treatment groups is suggestive of damage to the plasma membrane of these tissues at the cellular level, leading to increased efflux of these enzymes into the extracellular fluid (Huang et al., 2009).

Meanwhile, our results about the effects of Fenbendazole and Rafoxanide and their combination on serum protein, albumin, globulin level, the highest serum protein, albumin level and globuli level observed in group treated with Fenbendazole (37.5 mg/Kg) treated group after the 2nd week of experiment. Our results agreed with those of (Arise and Malomo, 2009) where they observed that, the level of serum protein increased with administration of Ivermectin + Albendazole (Ricobendazole) or fenbendazole than that of the administration of fenbendazole alone.

Also, our results on the effects of Fenbendazole and Rafoxanide and their combination on sperm count level, cleared that, the lowest sperm count level and sperm motility percentage observed in Fenbendazole (37.5 mg/Kg) treated group after 2nd weeks of the experiment. Followed by Rafoxanide (7.5 mg/Kg) orally, after 2nd weeks of experiment followed by combination of Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) especially after 4th of experiment.

Our results about the effects of Fenbendazole and Rafoxanide and their combination on sperm abnormalities level, cleared that, the higher sperm abnormalities level observed in the group treated with Fenbendazole (37.5 mg/Kg) treated group especially after the 4th week of the experiment. Followed by the group treated with combination of Fenbendazole (37.5 mg/Kg) + Rafoxanide (7.5 mg/Kg) especially after 4th of experiment.

The histopathological results supported this findings as it showed that, in the Fendbendazole treated group, the testes of the treated rats revealed congestion of the testicular blood vessels and interstitial edema. Multifocally, the lining epithelial cells of small numbers of seminiferous tubules exhibited degenerative changes characterized by swollen pale vacuolated cytoplasm. The epididymis showed interstitial edema and normal histological structure of epididymal tubules. The seminal vesicles showed congested blood vessels, mild desquamation with vacuolar and hydropic degeneration of the lining epithelial cells. (Arise and Malomo, 2009).

Our results concluded that, using of Fenbendazole for treatment of helminthes parasites improved the index weight of accessary sex glands, tests index weight and epidedymis index weight. Also, the values WBCs, Hb, PCV, serum protein, albumin globulin, serum urea, serum creatinine level and serum observed in Fenbendazole treated group compared to the rafoxanide treated group or their combination. But it decreased sperm motility, sperm count in Fenbendazole and the highest sperm abnormalities percentage observed in the group treated with Fenbendazole. While, the rafoxanide improved sperm motility, count and less sperm abnormalities than fenbendazole or their combination. The histopathological results cleared that, there is a severe histological changes in the group treated with fenbendazole and rafoxanide compared to the control group and their combination.

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