**Alexandria Journal of Veterinary Sciences 2014, 42:36-48**
**ISSN 1110-2047, www.alexjvs.com**
**DOI: 10.5455/ajvs.162332**

# Pharmacodynamics Studies on Ricobendazole In Male Rats

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**Key words**

Ricobendzole – Fertility – Male rats – Biochemical changes

**ABSTRACT:**

The present study was conducted to study the effect of ricobendazole on male fertility, as well as its effects on the liver and kidney functions tests, some hematological parameters and histopathological changes. Forty-five mature male albino rats were used and divided into 3 equal groups. The first group was kept as control and subcutaneously injected with proplyene glycol (2 ml /kg b.wt.). The second group was received a single subcutaneous injection of ricobendazole at a dose of 7.5 mg/kg bwt. The third group was subcutaneously injected with 7.5 mg / kg bwt twice within 3 weeks interval. The obtained results showed that administration of ricobendazole as single or repeated dose 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 test values such as Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (AlP) were significantly increased. Moreover, administration of ricobendazole induced histopathological alterations in reproductive organs, liver and kidney. The adverse effects of ricobendazole was more pronounced in repeated dose group. Therefore, caution is required when using repeated doses of ricobendazole .

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## 1-INTRODUCTION

Ricobendazole is benzimidazole methyl carbamate, which called albendazole sulfoxide Albendazole sulfoxide is used as a broad-spectrum anthelmintic in veterinary medicine. It is the first metabolite of albendazole (Lanusse and Prichard, 1990; Formentini et al., 2005). After administration, albendazole sulfoxide is transformed into its major metabolite, albendazole sulfone by hepatic microsomal cytochrome P-450 (El-Amri et al., 1987).Among the anthelmintic agents, benzimidazoles present a broad spectrum of activity with high effectiveness and safety (Campbell, 1990), however administered during gestation, they have demonstrated teratogenic effects such as external, skeletal and vascular abnormalities (Cristofol *et al.*, 1997; Navarro *et al.*, 1998,1999; Teruel *et al.*, 2003).

A novel ricobendazole injectable solution 15% for subcutaneous administration is available in different countries. It is successfully used to control abomasal and intestinal nematodes and lung worms. A 5 mg/kg bwt dose rate is recommended to control larval 4$^{th}$ stage of *O. ostertagi*. The formulation is not recommended for control of liver flukes or tape worms. In Europe,ricobendazole is also approved for use in pheasants by feed administration (17 mg/kg during 3 days) and is mainly used for the control of ascarids and capillarid infection (Riviere and Papic, 2009).

Albendazole sulfoxide binds to and inhibits polymerization of beta-tubulin, which inhibits cytoplasmic microtubule formation and glucose uptake within the parasite leading to immobilization and death of adult worms and prevents hatching of eggs (Pearson and Guerrant, 1983; Del bruto, 1993; Magill et al., 2012).

Several studies suggest that the capacity of benzimidazolic drugs to bind with the tubulin of cellules is responsible for the toxic effects observed during the gestation (Delatour and Parish, 1986; Piscopo and Smoak, 1997). While albendzaole possesses a clear therapeutic effect, some pharmacokinetic studies indicate that albendazole sulfoxide is responsible for both anthelmintic and toxic effects [Delatour et al., 1981; Villaverde et al., 1995). Albendazole sulfoxide and sulfone dominate plasma profile and are the major metabolites in the urine. Albendazole sulfoxide is the pharmacological and embryo toxic active agent whereas the sulfone is inactive and nontoxic (Del Brutto et al., 1993; Cutter et al., 1997; Takayanagui et al., 1997).

Albendazole, as well as its pro-drug netobimin and other benzimidazoles are teratogenic in rats and other animal species inducing an increased number of resorptions, decreased fetal weight, as well as external and skeletal malformations (Fabre et al., 1989; Mantovani et al., 1995; Navarro, 1996). Parent drug albendzole and of two primary metabolites, the sulfoxide and the sulfone provoked a rise in mitotic index resulting from cell division blockage at the prophase or at the metaphase (ABZ metabolites) stage, and ABZ was more cytotoxic than its metabolites (Rolin et al., 1989)

The studies concerning effect of ricobendazole on male fertility are rather scarce (Insufficient data avalaible for this subject). So, this study was designed to determine some pharmacological actions of ricobendazole in male rats including its effect on reproduction. As well, its effects on some hepato=renal biochemical parameters and the histological changes in liver, kidney, testes, epididymis and accessory sex gland.

## 2-Materials and Methods

**2.1. Ricobendazole:** Ricomax® each 1ml contains ricobendazole 150 mg produced and supplied by PROVET Veterinary Products**.** All biochemical analytical kits and other chemicals were purchased from bio diagnostic ch.co.

**2.2 Experimental design:** Fourty-five mature male albino rats weighing from 160-190 gm b.wt. and (150-180 day old) were purchased from Alexandria Research Institute were used to study the effects of single and repeated doses of ricobendazole on blood picture, semen character, weight and histology of the reproductive organs as well as renal and hepatic functions. Rats were fed on bread, barely, corn, green leaves of vegetables and water *ad-libitum*.

The animals were divided into 3 equal groups, 15 rats each.

**The first group** was given propylene glycol 2 ml/kg bwt subcutaneous injection, twice with 3 weeks intervals.

**The second group** was given 7.5 mg/kg bwt of ricobendazole subcutaneous injection once. These doses were calculated according to oral dose recorded by **Teruel *et al*., (2009).**

**The third group** was given 7.5 mg/kg bwt of ricobendazole subcutaneous injection, twice with 3 week intervals.

Five rats from each group were sacrified after 2 weeks, 4 weeks and 8 weeks from the beginning of drug administration. Blood,

tissues and semen were obtained as the follow:

**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:**After sacrification of rats, 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 progressively motility percentage were 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 was observed under high power lens power 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 vigrously. 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.

**c-Hormonal assay:** Serum testosterone was determined using an enzyme immunoassay kit Immunometrics Ltd., London, UK) according to Demetrious, (1987).

**2.5. Weight of Internal Body Organs:**

The animals were then sacrificed after 2weeks,4weeks and 8 weeks from administration then the testes, epididymis and accessory sex organs were dissected out, grossly examined and weighed. The index Wt (I.W) of each organ was calculated as described by Matousek, (1969).

$$\text{Index weight (I.W)} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

**2.6. Biochemical studies:** The collected sera were used to investigate the effect of ricobendazole on hepatic and renal functions. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was estimated according to Gella et al. (1985). Serum alkaline phosphatase (ALP) was measured according to the method described by Kind and King (1954).Serum creatinine was measured photometrically according to Henry(1974). Enzymatic determination of serum urea according to according to Pattons and Crouch (1977) was done.

***2.*7. Hematological studies:** Red blood cells, white blood cells count, hemoglobin level and packed cell volume value were measured by Mindary hematology analyzer BC-2300 (diamond diagnostic co. USA).

**2.8. Histopathlogical studies:**

Five rats from each group were decapitated. The testis, epididymis and prostate gland were dissected out grossly examined, dried by filter paper and weighed separately. Moreover, tissues from the livers and kidneys of all rats were collected. All specimens were preserved in 10% neutral buffer formalin solution. These fixed specimens were dehydrated in ascending grades of ethyl alcohol 70, 86, 96, 100% cleared in chloroform and embedded in paraffin. Sections of five microtone thickness were prepared and stained by Hematoxyllin and Eosin stain ***(Culling, 1974).***

### 2.9. Statistical analysis

Statistical analysis was performed using the SAS computer program (SAS, 2002). The data were analysed using analysis of variance (ANOVA) with Duncan's multiple range test to compare treatment means at $P < 0.05$. All data are expressed as the mean ± standard error (SE).

## 3-RESULTS

### 3.1. Fertility studies:

**1- Reproductive organs index weight:**

It was found that subcutaneous administration of ricobendazole (7.5 mg/kg bwt) as single dose or repeated doses after 3 weeks from the beginning of administration showed that there was a significant decrease in weight of testes in both groups all over the experimental period compared with control. The reduction was more pronounced in repeated dose group at $8^{th}$ week of the experiment than other groups.while there was a significant decrease in the weight of epididymis in single and repeated dose group at $4^{th}$ and $8^{th}$ week of the experiment as compared with control .Moreover, there was a significant decrease in weight of accessory sex gland in single and repeated dose group at $8^{th}$ week of the experiment as compared with control (Table 1).

**2- Sperm motility%, sperm count, sperm abnormalities% and testosterone level:**

The results showed that subcutaneous administration of ricobendazole (7.5 mg/kg bwt) as single dose or repeated doses after 3 weeks from the beginning of administration at different periods of experiment induced a significant decrease in the progressive sperm motility % in single and repeated dose group all over the experimental period compared with control. The reduction is more pronounced in repeated dose group at $4^{th}$ week of the experiment than other groups. However, there was a significant decrease in sperm count in single and repeated dose group all over the experimental period compared with control. The reduction was more pronounced in repeated dose group at $4^{th}$ and $8^{th}$ week of the experiment than other groups.

The obtained data showed that there was a significant increase in sperm abnormalities in single and repeated dose group all over the experimental period compared with control. The reduction was more pronounced in repeated dose group at 4th and $8^{th}$ week of the experiment than other groups. Moreover, there was a significant decrease in testosterone level in single and repeated dose group at $4^{th}$ and $8^{th}$ week of experiment compared with control. The reduction was more pronounced in repeated dose group at $8^{th}$ week of the experiment than other groups (Table 2).

### 3.2. Biochemical studies

There was a significant increase in some liver enzyme after subcutaneous administration of ricobendazole (7.5 mg/kg bwt) as single dose or repeated dose after 3 weeks from the beginning of administration. The obtained data showed that there was a significant increase in serum ALT in single and repeated dose group all over the experimental period compared with control. The increase was more pronounced in repeated dose group at

8th week of the experiment than other groups. There was a significant increase in serum AST in single and repeated dose group all over the experimental period compared with control.

**Table1.** Effect of subcutaneous administration of ricobendazole (7.5 mg/kg bwt) as single or repeated doses after 3 weeks from the beginning of administration on the index weight of reproductive organs at different periods in adult male rats

| parameters | testis index weight | | | Epididymis index weight | | | Accessory sex glands index weigh | | |
|---|---|---|---|---|---|---|---|---|---|
| Time / Group | 2nd week | 4th week | 8th week | 2nd week | 4th week | 8th week | 2nd week | 4thWeek | 8t week |
| Control | 1.56 ±0.01$^{a}$ | 1.61 ±0.02$^{a}$ | 1.57 ±0.04$^{a}$ | 0.72 ±0.05$^{a}$ | 0.82 ±0.06$^{a}$ | 0.72 ±0.02$^{a}$ | 0.78 ±0.02$^{a}$ | 0.78 ±0.02$^{a}$ | 0.72 ±0.07$^{a}$ |
| Ricobendazole (single dose) | 1.42 ±0.03$^{b}$ | 1.37 ±0.06$^{b}$ | 1.31 ±0.09$^{b}$ | 0.85 ±0.18$^{a}$ | 0.50 ±0.03$^{b}$ | 0.54 ±0.04 $^{b}$ | 0.82 ±0.05$^{a}$ | 0.59 ±0.08$^{a}$ | 0.37 ±0.03$^{b}$ |
| Ricobendazole (repeated dose) | 1.42 ±0.02 $^{b}$ | 1.40 ±0.06$^{b}$ | 1.21 ±0.07$^{c}$ | 0.70 ±0.04$^{a}$ | 0.56 ±0.07$^{b}$ | 0.52 ±0.03 $^{b}$ | 0.74 ±0.02$^{a}$ | 0.69 ±0.04$^{a}$ | 0.46 ±0.01$^{b}$ |

**Values are expressed as mean ± standard error. (n=6) Values at same column carrying the same letters are not significantly different ($p \leq 0.05$)**

**Table 2.** Effect of subcutaneous administration of ricobendazole (7.5 mg/kg bwt) as single dose or repeated dose after 3 weeks from the beginning of administration on fertility parameters at different periods in adult male rats.

| Parameter | Sperm motility (%) | | | sperm count (×106/ml | | | Sperm abnormalities (%) | | | Testosterone (ng/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | 87.00± 1.63 $^{a}$ | 90.00± 1.39 $^{a}$ | 92.6± 1.09 $^{a}$ | 295.0 ± 2.89 $^{a}$ | 280 ± 2.58 $^{a}$ | 282. ± 5.26 $^{a}$ | 8.00± 0.37 $^{b}$ | 9.67 ±0.42 $^{c}$ | 11.00± 0.73 $^{c}$ | 3.30 ±0.26 $^{a}$ | 3.30 ±0.26 $^{a}$ | 2.50 ±0.06 $^{a}$ |
| Ricobendazole (single dose) | 79.50 ±0.76 $^{b}$ | 80.00 ±0.58 $^{b}$ | 73.33 ± 1.38 b | 196. ± 6.91 $^{b}$ | 145.00± 3.42 $^{b}$ | 207. ± 4.28 $^{b}$ | 35.17± 1.49 $^{a}$ | 30.00 ±0.86 $^{b}$ | 38.33± 2.12 $^{b}$ | 3.62 ±0.4 $^{a}$ | 1.62 ±0.39 $^{b}$ | 1.62 ±0.15 $^{b}$ |
| Ricobendazole (repeated dose) | 80.50 ±0.92 $^{b}$ | 70.00 ±0.58 $^{c}$ | 75± 1.15 $^{b}$ | 193± 6.04 $^{b}$ | 93.61± 8.45 $^{c}$ | 102.4± 8.06 $^{c}$ | 34.83± 0.75 $^{a}$ | 42.00 ±1.06$^{a}$ | 48.50± 1.82 $^{a}$ | 3.30 ± 0.75 $^{a}$ | 1.30 ±1.06 $^{b}$ | 1.09 ±1.82 $^{c}$ |

Values are expressed as mean + standard error. (n=6)
Values at same column carrying the same letters are not significantly different ($p \leq 0.05$)

**Table3.** Effect of subcutaneous administration of ricobendazole (7.5 mg/kg bwt) as single dose or repeated dose after 3weeks from the beginning of administration on liver enzymes) level at different periods in adult male rats.

| Parameter | 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 | 37.00± 1.37 $^{b}$ | 37.00± 1.97 $^{b}$ | 32.67± 0.49 $^{c}$ | 104.67± 4.72$^{b}$ | 94.17± 0.70 $^{b}$ | 96.50± 1.86 $^{b}$ | 85.00 ±2.58$^{b}$ | 90.33 ±4.84$^{c}$ | 88.00 ±0.58$^{c}$ |
| Ricobendazole (single dose) | 67.17± 2.51 $^{a}$ | 51.00± 2.80 $^{a}$ | 65.50± 2.62 $^{b}$ | 141.83± 1.76 $^{a}$ | 127.17± 2.17 $^{a}$ | 134.00± 0.97 $^{a}$ | 135.83± 18.90$^{a}$ | 116.17 ±2.02$^{b}$ | 149.33± 20.62$^{b}$ |
| Ricobendazole (repeated dose) | 67.00± 2.58 $^{a}$ | 57.00± 2.07 $^{a}$ | 83.00± 0.52 $^{a}$ | 142.17± 1.17 $^{a}$ | 132.83± 2.66 $^{a}$ | 136.17± 3.37 $^{a}$ | 136.17 ±3.52$^{a}$ | 138.00 ±0.77$^{a}$ | 200.67 ±5.96$^{a}$ |

Values are expressed as mean ±standard error (n=6).
Values at same column carrying the same letters are not significantly different ($p \leq 0.05$)

**Table 4.** Effect of subcutaneous administration of Ricobendazole (7.5 mg/kg bwt) as single dose or repeated dose after three weeks from the beginning of administration on kidney function at different periods in adult male rats.

| Parameter | Urea (mg/dl) | | | Creatinine (mg/dl) | | |
|---|---|---|---|---|---|---|
| Time / Group | 2nd week | 4th week | 8th week | 2nd week | 4th week | 8th week |
| Control | 25.00±0.82$^{a}$ | 24.67±0.56$^{b}$ | 24.17±0.31$^{a}$ | 0.62±0.03$^{a}$ | 0.45±0.04$^{b}$ | 0.52±0.03$^{a}$ |
| Ricobendazole (single dose) | 25.17±1.22$^{a}$ | 23.00±0.58$^{b}$ | 26.17±0.60$^{a}$ | 0.68±0.03$^{a}$ | 0.48.±0.02$^{b}$ | 0.50±0.00$^{a}$ |
| Ricobendazole (repeated dose) | 24.00±0.93$^{a}$ | 28.00±0.73$^{a}$ | 22.83±0.95$^{a}$ | 0.66±0.04$^{a}$ | 0.67±0.03$^{a}$ | 0.48±0.04$^{a}$ |

Values are expressed as mean ± standard error (n=6).
Values at same column carrying the same letters are not significantly different ($p \leq 0.05$)

**Table 5.** Effect of subcutaneous administration of Ricobendazole (7.5 mg/kg B. wt) as single dose or repeated dose after three weeks from the beginning of administration on total protein, albumin and globulin level at different periods in adult male rats.

| Parameter | Total protein (g/dl) | | | Albumin (g/dl) | | | Globulin (g/dl) | | |
|---|---|---|---|---|---|---|---|---|---|
| Time / Group | 2nd week | 4th week | 8th week | 2nd week | 4th week | 8th week | 2nd week | 4th week | 8th week |
| Control | 7.25 ±0.08$^{a}$ | 6.95 ±0.12$^{a}$ | 7.12 ±0.11$^{a}$ | 3.42± 0.06$^{a}$ | 3.40± 0.06$^{a}$ | 3.50± 0.06$^{a}$ | 3.83± 0.10$^{a}$ | 3.55± 0.13$^{a}$ | 3.62± 0.08$^{a}$ |
| Ricobendazole (single dose) | 5.77 ±0.17$^{b}$ | 5.23 ±0.10$^{b}$ | 6.23 ±0.18$^{b}$ | 2.70± 0.11$^{b}$ | 2.47± 0.12$^{b}$ | 2.67± 0.34$^{b}$ | 3.12± 0.19$^{b}$ | 2.77± 0.04$^{b}$ | 3.57± 0.23$^{a}$ |
| Ricobendazole (repeated dose) | 5.88 ±0.27$^{b}$ | 5.45 ±0.09$^{b}$ | 6.57 ±0.07$^{b}$ | 2.70± 0.18$^{b}$ | 2.65± 0.09$^{b}$ | 2.83± 0.11$^{b}$ | 3.13± 0.28$^{b}$ | 2.80± 0.17$^{b}$ | 3.74± 0.17$^{a}$ |

Values are expressed as mean ± standard error (n=6).
Values at same column carrying the same letters are not significantly different ($p \leq 0.05$)

**Table 6.** Effect of subcutaneous administration of Ricobendazole (7.5 mg/kg bwt) as single dose or repeated dose after 3weeks from the beginning of administration on hematological parameters count at different periods in adult male rats.

| Parameter | PCV % | | | RBCs count (×10$^{6}$/cmm) | | | Hb (g/dl) | | | WBCs count (×10$^{3}$/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 | 38.00± 0.40$^{a}$ | 39.00 ±0.52$^{a}$ | 39.33 ±0.49$^{a}$ | 6.23 ±0.15$^{a}$ | 6.17 ±0.10$^{a}$ | 6.03 ±0.08$^{a}$ | 12.27± 0.28$^{a}$ | 12.30± 0.57$^{a}$ | 12± 0.08$^{a}$ | 12.00± 0.76$^{a}$ | 12.23 ±0.67$^{a}$ | 13.22 ±0.37$^{a}$ |
| Ricobendazole (single dose) | 33.89± 0.40$^{b}$ | 36.00± 1.38$^{a}$ | 39.00 ±0.45$^{a}$ | 5.73 ±0.18$^{b}$ | 5.95 ±0.26$^{a}$ | 6.00 ±0.06$^{a}$ | 11.17± 0.26$^{a}$ | 11.05± 0.46$^{a}$ | 11.95± 0.14$^{a}$ | 11.18± 1.14$^{a}$ | 10.93 ±0.59$^{a}$ | 13.03± 0.41$^{a}$ |
| Ricobendazole (repeated dose) | 34.17± 0.79$^{b}$ | 35.50± 0.96$^{b}$ | 38.33± 0.42$^{a}$ | 5.65± 0.58$^{b}$ | 5.66± 0.12$^{b}$ | 5.92 ±0.05$^{a}$ | 11.13± 0.60$^{a}$ | 9.98± 0.36$^{b}$ | 11.47± 0.36$^{b}$ | 11.58± 0.36$^{a}$ | 8.68 ±0.32$^{b}$ | 11.97± 1.70$^{a}$ |

Values are expressed as mean ± standard error (n=6).
Values at same column carrying the same letters are not significantly different ($p \leq 0.05$)

**4. Histopathological examination:**

The microscopical examination of testicular tissue of both treated groups allover the experimental period showed congestion of the testicular blood vessels and interstitial capillaries. The interstitium was markedly expanded by homogenous eosinophilic material (edema) and degenerative changes of the lining epithelial cells of moderate number of seminiferous tubules characterized by swollen pale vacuolated cytoplasm. Moreover, testis of rat of received subcutaneous repeated dose of ricobendazole and killed 4 weeks and 8 weeks post 1st administration showed degenerated germ cells accompanied by incomplete spermatogenesis and absence of spermatozoa in the lumen of seminiferous tubules (Fig 1).regarding to prostate gland there was congested blood vessels with cystic dilatation of some prostate glands lined by attenuated epithelium was also detected with mild interstitial edema experiment in both treated groups.The seminal vesicles in both treated groups showed hyperplastic changes of their lining epithelium and congestion of blood vessels and lymphocytic cellular infiltration infiltration.in both groups all over the experiment. Moreover, the seminal vesicles of rats received repeated dose of ricobendazole at 4th and 8th of experiment showed desquamation of glandular epithelium with presence of cellular debris in lumen of some seminal gland (Fig 2). The epididymis showed normal histological appearance of their tubules in both groups all over experiment except at $4^{th}$ and $8^{th}$ week in repeated dose group Epididymis showed sperm giant cell inthe lumen of an epididymis tubule (Fig 3). The microscopical examination of the livers of both treated groups revealed congestion of portal blood vessels and vacuolar and hydropic degeneration of hepatocytes characterized by swollen pale vacuolated cytoplasm of both treated groups allover the experimental period. More over rats treaed with repeated dose of ricobendazole the liver showed congestion of centeral vein together with coagulative necrosis of some hepatocytes by pyknosis of their nuceli and homogenous deep esinophilic cytoplasm were occasionally seen in repeated dose group at 4th week of experiment (Fig 4).

The examined kidneys revealed congestion of the renal blood vessels and intertubular capillaries. The perivascular interstitium of renal cortex was expanded by increased clear few number of mononuclear inflammatory cells The renal cortex showed degenerative changes of some renal convoluted tubules evidenced by coagulative necrosis, vacuolar and hydropic degeneration of the lining epithelium of the proximal and distal convoluted tubules in all treated rats allover the experimental period. The glomeruli revealed hyper segmentation of glomerular tuft with cytoplasmic vacuolization of endothelial cells or shrinkage and necrosis of glomerular tufts only in repeated dose group at $4^{th}$ week and $8^{th}$ week of experiment (Fig 5).

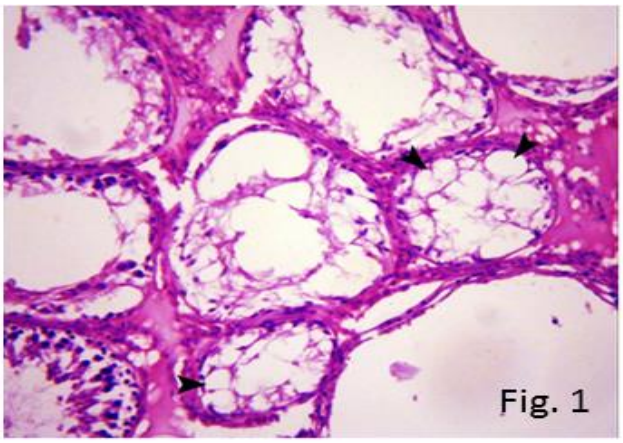


**Fig. 1**: Testis of rat received subcutaneous repeated dose (7.5 mg/kgbwt) of ricobendazole after three weeks from 1st administration killed 4 weeks post 1st administration showing degenerated germ cells (arrow head) accompanied by incomplete spermatogenesis and absence of spermatozoa in the lumen of seminiferous tubules. H&E stain × 400.

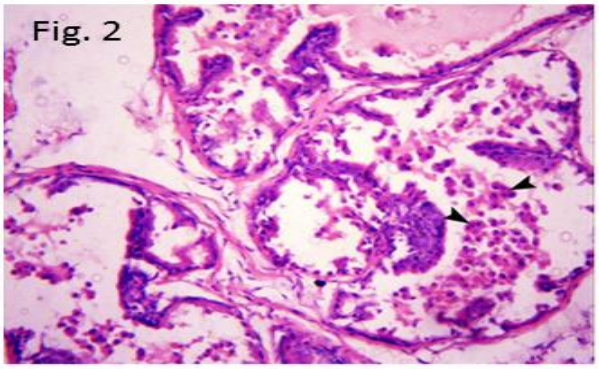


**Fig. 2**: Seminal vesicle of rat subcutaneous repeated dose (7.5 mg/kgbwt) of ricobendazole after three weeks from 1st administration and killed 4 weeks post 1st administration showing necrosis and desquamation of the lining epithelium (arrow head) of some seminal glands. H&E stain × 200.

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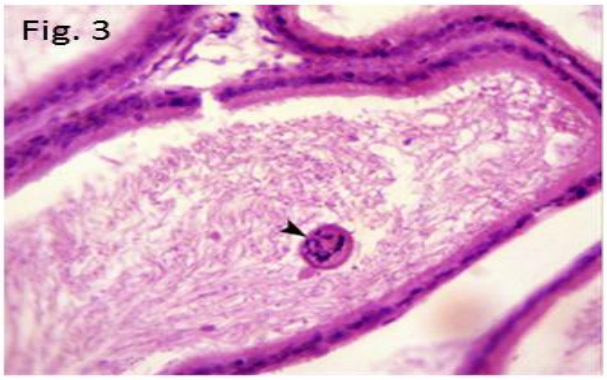


**Fig. 3**: Epididymis of rat administered subcutaneous repeated dose (7.5 mg/kgbwt) of ricobendazole after three weeks from 1st administration and killed 4 weeks post 1st administration showing sperm giant cell (arrow head) in the lumen of an epididymis tubule. H&E stain × 400

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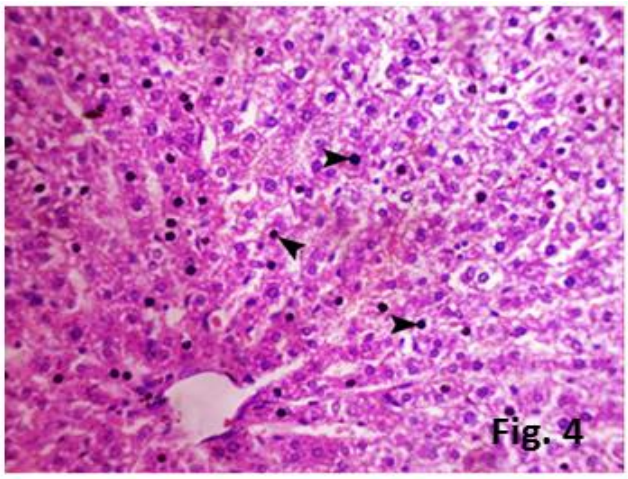


Fig. 4: Liver of rat administered subcutaneous repeated dose (7.5 mg/kgbwt) of ricobendazole after three weeks from 1st administration and killed 4 weeks post 1st administration showing coagulative necrosis of some hepatocytes evidenced by pyknosis of the nuclei (arrow head). H&E stain × 400.

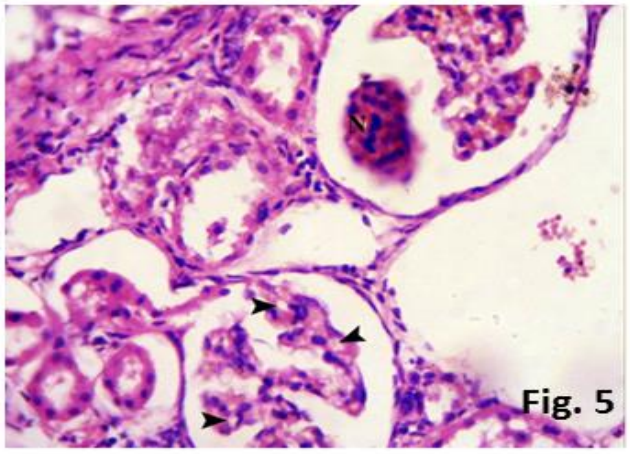


**Fig.5:** Kidney of rat administered subcutaneous repeated dose (7.5 mg/kg bwt) of ricobendazole after three weeks from 1st administration and killed 4 weeks post 1st administration showing hypersegmentation of glomerular tuft with cytoplasmic vacuolization (arrow head) and necrosis (N) of endothelial cells of the tuft. H&E stain ×400.

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## 5. DISCUSSION

In the present study, the effect of ricobendazole at single and repeated dose levels on some reproductive parameters was examined in addition; some biochemical parameters and hematological parameters were also investigated. Measurements were made at different periods from onset of drug administration to follow up the induced effects of the drug on the reproductive, hepatic and renal functions.

The duration of the present study lasts for 8 weeks to cover complete spermatogenic cycle in rats which ranges from 48 – 52 days (Clermont and Harvey, 1965).The ricobendazole administration induced a significant decrease in weights of the testes, epididymis and accessory sex organs. These findings may be due to decrease of testosterone hormone level which showed a significant decrease in its level after 4$^{th}$ week from subcutaneous administration of ricobendazole at dose level 7.5 mg/kg bwt as single dose or repeated dose after 3 weeks from 1st injection, and still decreased after 8$^{th}$ week from drug administration. The reported findings are inagreement with those recorded by El sukary et al. (2010) who reported oral administration of albendazole induced significant decrease in the weights of testes and epididymis and accessory glands.

The reduced sperm content implies an adverse effect on spermatogenesis in rats that received Ricobendazole. Impaired sperm motility in treated rats is indicatives to a defect in the acquisition or maintenance of motility. Ricobendazole may alter the epididymal secretory products or has a direct action on sperm motility or morphology. These results are supported by those of Alexander (1978) who reported a reduction in the number of sperm and motility due to inhibition of both spermatogenesis and sperm maturation. The reported findings are agreement with those recorded by El sukary et al. (2010) who revealed that there was adecrease in progressive sperm motility after two and four weeks from oral drug administration using 30 and 60 mg albendazole/kg bwt. Also there was a significant decrease in sperm cell count and increase in sperm abnormalities after four and 8 week from administration using 60 mg albendazole/ kg bwt.

These alterations in the fertility were confirmed by our histopathological findings in reproductive organs which represented by congestion, degenerative changes, also showed in complete spermatogenesis and absence of spermatozoa in testes of treated rats.

The obtained data revealed a significant increase in activities of ALT, AST and ALP in the serum of the treated rats. ALT occurs exclusively in the liver, but only in the cytoplasm of parenchymal cells.) the level of

ALT and ALP enzymes is increased following liver damage. (Doxy 1971; Hoe and Wikinson, 1973). Accordingly, the mentioned results of enzymes after ricobendazole administration in both treated groups are attributed to damage of the liver. This conclusion is supported also by the finding reported by coles (1974) and Schiff (1969) who mentioned that increased serumtransaminases and ALP activities observed frequently in various hepatocellular the finding reported by coles (1974) and Schiff (1969) who mentioned that increased serum transaminases and ALP activities observed frequently in various hepatocellulardamage.

These results are compatible with by Arise and Malomo (2009) recorded that activities of ALP, ACP, LDH, AST, ALT, $NA^{+}$ $K^{+}$ ATPase and $Ca^{+2}$ $Mg^{+2}$ ATPase of liver and kidney were significantly altered in a study where albino rats were administered 15 mg/kg bwt of albendazole daily for fifteen days. These observations were due to deranged membrane structure and functions. These results were confirmed by El sukary et al. (2010) who mentioned that albendazole induced significant increase in ALT activity on administration of both doses after 4 or 8 weeks from drug administration while AST showed increase in its activity after 8 weeks from drug administration using 60 mg albendazole/kg bwt.

These results were confirmed by histopathological findings (congestion of portal blood vessels, vacuolar and hydrooic degeneration of hepatocytes) support the liver damage and subsequently increased liver enzymes.

It was apparent from the present study that ricobendazole subcutaneous at dose level 7.5 mg/kg bwt as single or repeated dose after 3 weeks from first injection to adult male rats induced significant increase of urea and creatinine levels only at $4^{th}$ week in repeated dose group These elevation is reflect the state of glomerular filtration and indicate kidney damage. These results supported by Arise and Malomo (2009) who mentioned that administration of albendazole led to significant increase in serum urea and creatinine in a study where albino rats were administered 15 mg/kg bwt of albendazole daily for fifteen days after which venous blood, liver and kidney were collected. the increased serum urea and creatinine were due to damage membrane structures and functions.while the results are Incompatabile with El Sukary et al. (2010) who demonstrated that the level of seum urea increased all over experiment period of experiment, with both doses of albendazole. While there was significant increase in level of serum creatinine after 4weeks from drug administration using 30 and 60 mg albendazole/kg bwt. The difference between the previous study in comparison with the present may be due to the differences in the drug dose, frequency and mode of administration.

The histopathological examination of kidneys (congestion of the renal blood vessels and intertubular capillaries.cloudy swelling, vacuolar and hydropic degeneration of the lining epithelium of the proximal and distal convoluted tubules and coagulative necrosis of renal tubules) confirmed the kidney damage and subsequently increased urea and creatinine levels.

The obtained results demonstrated that subcutaneous administration of ricobendazole at dose level 7.5 mg/kg bwt induced significant decrease in RBCs count and PCV at 2nd week from drug administration in single dose group, and at $2^{nd}$ and $4^{th}$ week from drug administration in repeated dose group. While there were insignificant changes in RBCs count and PCV after 8weeks from the onset of drug administration.Also induced significant decrease in WBCs count and Hemoglobin level in repeated dose group at $4^{th}$ week from drug administration. These result are incompatible with EL-Sukary et al. (2010) who demonstrated that oral administration of albendazole in male rats of both doses (30 mg and 60 mg/ kg B.wt) induced insignificant changes on hemoglobin level, RBCs count, packed cell volume and WBCs count all over the period of experiment and also incompatible with those reported by Gulani et al. (2007) who mentioned that the

routine administration of intestinal anthelmintic agents results in marginal increase in hemoglobin (1.71 g/l). The difference between the previous studies in comparison with the present may be due to the differences in healthy state and the drug dose and mode of administration.

It could be concluded that attention should be paid on using repeated doses of ricobendazole that's due to the abnormal semen character and the harmful effects that can be induced on liver and kidney functions.

## 6. REFERENCES


Alexander, E.P., Newton, G.G., Bronwen, L.1960. Animals Models for research on Contraception and Fertility, Harper and Row. P. 106:326.

Arise, R.O., Malomo, S.O. 2009. effects of ivermectin and albendazoleon some liver and kidney function indicies in rats.African Journal of Biochemistry research, VoL.3 (5), p.190-197.

Berdan, H., Fuquay, J. 1980. Applied Animal Reproduction. Reston Publishing Co., Virginia, P. 158 – 160

Campbell, W. C. 1990. Benzimidazole: veterinary uses. *ParasitoL Today., 6*:130-3.

Clermont, Y., Harvey, *S.C.1965.*Duration of the somniferous epithelium of normal, hypophysectomized and hypophysectomized hormone treated albino-rats . Endocrinal. 79; 80-89.

*Coles, E.H.1974.*Veterinary Clinical Pathology. Pp. 213, W.B. Saunders Company, Philadelphia, London, Toronto.

Cristofol, C., Navarro, M., Franquelo, C.,Valladares, J. P.; Carretero, A.; Ruberte, J., Arboix, M. 1997. Disposition ofnetobimin, albendazole and its metabolites in thepregnant rat: Developmental Toxicity. Toxicol. Appl.Pharmacol., 144:56-61.

Cuetter,A.C., Garcia-Bobadilla, J., Guerra, L.G., Martinez, F.M., Kaim, *B. 1997.* Neurocysticercosis: focus on intraventricular disease. Clin Infect Dis. 24:157–164.

Culling, G.F. 1974. Handbook of Histological and Histochemical Techniques 3rd Ed. Butterworth, London, Boston :214.

Del Brutto OH, Sotelo J, Roman GC. 1993): Therapy for neurocysticercosis: areappraisal. Clin Infect Dis.17:730–735.

Del Bruto, O.H., 1993. cysticercosis. New Engl. J. Med. 329, 913.

Delatour, P., Parish, R.c., Gyurik, RJ. 1981. Albendazole: A comparison of relay embr-yotoxicity with embryotoxicity of individual metabolites. Ann. Rech. Vet. 12: 159-167.

Delatour, P., Parish, R. 1986. Benzimidazole anthelmintics and related compounds: Toxicity and evaluation of residues. In Drug Residues in Animals (Rico AG. ed.), p. 175-303. Academic Press, New York.

Demetrious, J.A. 1987. Testosterone in methods. In Clinical Chemistry Tech, A.G .and Kapalan, L.A. 2nd Ed. CVMOS Co., pp.268.

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

El-Amri, H.S., Fargetton, X., Delatour, P., Batt,A.M., 1987. Sulphoxidation of albendazole by the FAD-containing and cytochrome P-450 dependent mono-oxygenases from pig liver microsomes.Xenobiotica, 17(10), 1159-1168.

EL-Sukary,N.A., EL-sawy,A.F., EL-Ashmawy,I.M.2010.pharmacological effects of albendazole on fertility of male rats.thesis in M.V.SC.in fac. Vet med. Alex. Univ.

Fabre, J. M., Berthelot, X., Ferney, J. 1989. Embryotoxicite´ des antiparasitaires chez les ovins: Observation clinique. Rev. Me´d. Ve´t,. 140, 1089–1095.

Formentini, E.A., Mestorino, N., Errecalde, J.O. 2005. pharamacokinetics of Ricobendazole after its intravenous , intraruminal and subcutaneous administration in sheep. Vet Res Commun. 29(7):595-608.

Gella, F.J., Olivella, T., Cruz Pastor, M.,Arenas, J., Moreno, R., Duraban, R., Gomez, J.A. 1985. A simple procedure for routine determination of aspartate aminotransferase and alanine transferase with pyridoxal phosphate. Clin. Chem. Acta; 153:244-247.

Gulani,A., Nagpal, J., Osmond,C., Sachdev,H.P. 2007. Effect of administration of intestinal

anthelminitic drugs on haemoglobin :systemic review of randomized controllef trials.BMJ. 334:1095.

Gyurik, R. J., Chow, A. W., Zaber, B., Bruner, E. L., Miller, J. A., Petka, L. A., Parish, R. C. 1981. Metabolism of albendazole in cattle, sheep, rats and mice. Drug Metabolism and Disposition 9, 503–508.

Henry, R.J (1974): Clinical Chemistry Priciples and Technics. 2nd Edition, Harper and Row. P. 525.

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

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

Lanusse, C. E., Prichard, R. K. 1990. Pharmacokinetic behaviour of netobimin and its metabolites in sheep.Journal of Veterinary Pharmacology and Therapeutics 13,170–178.

Magill, A.J., Strickland,T.G., Maguire,J.H. 2012. Hunter's Tropical Medicine and Emerging Infectious Disease, ninth edition. Elsevier Inc. p. 1088.

Mantovani,A., Ricciardi,C., Stazi,A.V., Macri,C. 1995. Effects observed on gestational day 13 in rat embryos exposed to albendazole. Reproductive Toxicology; 9:265–273.

Matousek, J. 1969. Effect on spermatogenesis in guinea pig ,rabbits and sheep after immunizatation with sexual fluid of bulls. J.Report. Fret., 19: 63 – 72.

Melhorn,H.,Armstong,M.P.2011.Encylopedic reference of parasitology :disease, treatment,therapy,2nd edition. Berline;heidberg:springer.p.372

Navarro, M. 1996. Efectos embrioto´xicos del netobimin en la oveja, la rata y el pollo. Doctoral Thesis, Publicacions UAB, Barcelona.

Navarro, M., Cristofol, C., Carretero, M., Arboix, M., Ruberte, J. 1998. Anthelmintic induced congenitalmalformations in sheep embryos using Netobimin. Vet.Rec., 142:86-90.

Navarro, M.1., Canut, L., Carretero, A., Cristofol, C., Pérez-Aparicio,F.J., Arboix, M., Ruberte, J. 1999. developemental toxicity in rat fetuses exposed to the benzimedazole netobimin, reported toxicol.13 (4): 295-302.

Pattons, C.J., Crouch, S.R. 1977).Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem., 49:464-469.

Pearson, R.D., Guerrant, R.L.1983. Praziquantel: a major advance in anti-helminthic therapy. Ann. Int. Med. 99,195-198.

Piscopo ,S., Smoak, I. 1997. Comparison of effects of albendazole sulphoxide on in vitro produced bovine embryos and ratsembryos. American Journal of Veterinary Research 58: 1038-1042.

Riviere,J.E., Papic.M.G. 2009. Veterinary Pharmacology and Therapeutics, ninth edition. Wiely black-well puplishing company. P. 1069.

Rolin ,S.; el Amri, H.S.; Batt, A.M.; Levy ,M.; Bagrel, D. and Siest ,G.(1989): Study of the in vitro bioactivation of albendazole in human liver microsomes and hepatoma cell lines. Cell Biol Toxicol.5(1):1-14.

Schiff, L. 1969. Disease of the liver.3rd Ed.J.B. Lipincott Com. Philadelphia. Montreal.

SAS, 2002. Statistical Analysis. User's Guide Statistics. SAS Institute Inc., Cary, North California, U.S.A.

Takayanagui, O.M., Lanchote, V.L., Marques, M.P.C., Bonato, P.S. 1997.Therapy for neurocysticercosis pharmacokinetic interaction of albendazole sulfoxide with dexamethasone. Ther. Drug Monit.19:51–55.

Teruel, M., Felipe, A., Solana, H., Sallovitz, J., Lanusse,C. 2003. Placental and fetal toxicity of albendazole sulphoxide in Wistar rats. Vet. Hum. Toxicol., 3:131-6.

Teruel, M.; García, V. & Catalano, R. 2009.Effects of albendazole sulphoxide on embryonic, foetal and placental parameters in Wistar Rats. Int. J. Morphol., 27(4):1147-1153.

Villaverde, C., Alvarez, S., Redondo, P. 1995. Small intestinal sulphoxidation of albendazole. Xenobiotica 25:433–441.