Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 59 (1): 107-118. Oct. 2018
DOI: 10.5455/ajvs.302507

Reproductive Effects of Clanobutin Sodium or Menbutone Diethanolamine

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Key words:
clanobutin sodium, menbutone diethanolamine, semen characters, testis, epididymis, pregnant female

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ABSTRACT

The present study was conducted to evaluate the effect of clanobutin sodium and menbutone diethanolamine each alone on some male fertility parameters of male goats. Moreover, the possible adverse effects of repeated administration of these drugs on the foeti of pregnant female rats and/ or induction of abortion were evaluated. Therefore, two experiments were carried out; the first was done on 15 apparently healthy mature un-castrated male goats of Zaraibi breed (1.5-2 year old) were divided into 3 equal groups. The 1st group: goats received basal ration and water without any treatment, the 2nd group: goats administered with clanobutin sodium (20 mg/kg b.wt in the 1st day and 10 mg/kg b.wt. in the 2nd day weekly for 8 weeks) and the 3rd group: goats administered with menbutone diethanolamine (10 mg/kg b.wt in the 1st day and 5 mg/kg b.wt. in the 2nd day weekly for 8 weeks). The second experiment was done on 45 pregnant female rats were divided into 3 equal groups. The 1st group of rats received basal ration and water, the 2nd group: rats administered with clanobutin sodium (40 mg/kg b.wt from 5th to 15th day of pregnancy period) and the 3rd group: rats administered with menbutone diethanolamine (20 mg/kg b.wt from 5th to 15th day of pregnancy period). The obtained results showed that administration of these drugs showed a deteriorating effect on male and female fertility parameters. Both drugs induced significant decrease in sperm concentration, sperm progressive motility, normal sperm %, in comparison with the control group of male goats all over the periods of the experiment. Also, the drugs induced a significant reduction in serum testosterone with significant increase on serum FSH and LH levels with harmful histophthological alterations in testis and epididymis Moreover, in the second experiment number, weights of foeti and number of live foeti on both uterine horns of pregnant female rats was significantly decreased with increased number of dead foeti on both clanobutin sodium and menbutone diethanolamine treated groups of rats in comparison with the control group. These results concluded that the use of both drugs on male or female animals intended for breeding must be avoided.

1. INTRODUCTION

Choleretics are substances that increase secretion of bile by the hepatocytes while, hydrocholeretics are drugs that stimulate the liver to increase out of bile of low specific gravity. Clanobutin sodium and menbutone diethanolamine are commonly used in veterinary practice as a result of their choleretic effect. (Talwar and Srivastava, 2002).Clanobutin sodium has been introduced as a choleretic and digestant agent for animals. It has been used in all domestic animals in which enhancement of digestion and all associated secretory processes are indicated. Calnobutin was reported to induce a demonstrable increase in the secretory activity of the exocrine digestive glands and in the excretion of the bile (Maciolek, 1975).

Menbutone [beta-(1-methoxy-4-naphthoyl) propionic acid or genabilic acid] is a choleretic compound which is a specific stimulant of exocrine glands of digestive tract; digestive tonic and choloretic in bovine, ovine, porcine and equine species (Symonds,. 1982). It is appetite stimulant used to restore the appetite and normal digestion in post-operative therapy, adjuvant

therapy in toxic conditions, pancreatic failure, bile stasis and osteoporosis and dietary error as in the obese dogs (Burridge, 1983). Menbutone helps in any course affecting the digestive system as diarrheas, anorexia and gastroenteritis. After being injected in the body, it increases biliary, pancreatic and peptic secretion by 2 to 5 times compared with the normal levels of these secretions (Ackerman, 2007). The reproductive toxicity of both drugs was not fully discussed by any previous researches nor by the manufacturing companies, therefore the present work was done to evaluate their effects on some male and female fertility parameters and their possible histopatholgical alterations on male goats as an essential requirement of the clinical pharmacological and therapeutic evaluation for such drugs commonly used in veterinary practice as described by Laurence and Bennett (1980).

2. MATERIALS AND METHODS

2.1. Drugs, chemicals and instruments:

Clanobutin sodium (Bykahepar ®) produced by Schering-Plough Co., USA. Menbutone diethanolamine (Genabil®), produced by Boehringer Ingelheim Animal Health Co., Ingelheim am Rhein, Germany. All the diagnostic kits used for assaying hormonal level were obtained from Pishtaz Teb Diagnostics European authorized representative JTC Diagnosemittel UG Schulweg 8D-34516 Voehl /Germany using Microplate reader with a 450 nm or 650 nm filter. Semen analysis was carried out using Computer Assisted Semen Analysis (CASA) (MeCASA 8000) according to the method described by Amann and Waberski (2014).

2.2. Animals:

The first experiment: fifteen apparently healthy mature male goats of Zaraibi breed free from any parasitic infestation (21-30 kg B.Wt and 1.5-2 year old) were used. Goats were divided into three equal groups:
The first group (control group): goats received basal ration and water without any treatment,
The second group: goats administered intramuscularly with clanobutin sodium (20 mg/kg b.wt in the first day and 10 mg/kg b.wt. in the second day once weekly for 8weeks) (the recommended manufacturing dose)
The third group: goats administered intramuscularly with menbutone diethanolamine (10 mg/kg b.wt in the first day and 5 mg/kg b.wt. in the second day once weekly for 8 weeks) (the recommended manufacturing dose).

All groups were fed on the same concentrate ration prepared from a corn-soybean meal according to NRC (1994) with roughages**.**

The second experiment: forty five pregnant female rats were divided into three equal groups employed as follows:
Group 1: pregnant rats were left as a control group.
Group 2: Repeated i.m administration of clanobutin sodium (once daily) (40 mg/kg b.wt.) to proven pregnant rats starting from 5th day to 15th day from gestation period as described by Berchtold et al., (1980) was carried out.
Group 3: Repeated i.m administration of menbutone diethanolamine (once daily) (20 mg/kg b.wt.) to proven pregnant starting from 5th day to 15th day from gestation period *(Paget and Barnes, 1964)* was carried out.

3. Samples collections

3. 1. Semen samples:

Semen samples were collected on the 2nd, 4th and 8th week from the beginning of treatment to determine the effects of drugs administration on semen characters by electro-ejaculation. The procedure of electro-ejaculation was carried out using the protocol described by Jimenez-Rabadan et al. (2012). Animals were laid in a lateral recumbent position, prepucial area was washed and the rectum was cleaned of feces then the probe, pre-coated with an ultrasound gel (carboxymethyl cellulose) to improve electrical contact, was inserted into the rectum and located to the brim of the pelvis. Semen was collected in a pre-warmed 15-ml plastic tubes (Falcon™) connected to a pre-warmed small funnel. Immediately after collection, each ejaculate was transferred to a water bath maintained at 37°C prior to evaluation (Parkinson, 2009)

3. 2. Blood Samples:

Blood samples were collected from the jugular vein without anticoagulant from control and treated male goats on the 2nd, 4th and 8th week from the beginning of treatment (to determine the effects of drugs on hormonal levels of FSH, LH and testosterone hormone).The tubes were left in slope position to clot in room temperature. The tubes were centrifuged at 3000 r.p.m for 15 minutes and clear serum samples were carefully separated then transferred into dry clean epindorff's and kept frozen at -20 ^{o}C until used for Enzyme-Linked Immunosorbent Assay (ELISA) for hormonal determination.

3. 3. Histopathological samples:

At the 8th week of the experiment, all male goats were castrated to collect testis and epididymis from all

treated and control male goats to detect histopatholgical alterations caused by both drug administration. Castration process was carried out by **open method castration** in which all scrotum layers were surgically opened and the spermatic cord (including spermatic nerve , spermatic artery and ducts deferens) was tied and then cut to separate the testis and epididymis (Fubini and Ducharme 2017). Both organs from all control and treated male goats were individually weighted and kept on formalin10% until undergo histopathological studies.

4. Methods:

A. Experiments on un-castrated male goats:

1. Fertility studies on male goats:

1.a. The sperm motility, sperm cell concentration and sperm morphology:

The sperm motility, sperm cell concentration and sperm morphology were estimated by using Computer Assisted Semen Analysis (CASA) (MeCASA 8000). The system follows W.H.O strict criteria for motility patterns & Kruger strict criteria for morphology. A small drop of diluted semen (diluted with 2.9% Sod. Citrate dehydrate solution) was placed on glass side pre-warmed at 37°C and covered by a clean cover slip, then examined by Computer Assisted Semen Analysis (CASA) (MeCASA 8000).(Amann and Waberski, 2014).

1.b. Organ Weight index:

At the end of the experiment all male goats from each control and treated groups were castrated, testis and epididymis were collected and weighted. Organ weight index was calculated by the method described by Matousek (1969*)* as following:

Organ weight index = Organ weight/live body weight x 100

1.c.Enzyme-Linked Immunosorbent Assay (ELISA) for hormonal determination (FSH, LH and testosterone):-

This is an ELISA for the quantitative analysis of hormonal levels in biological fluid. This test kit operates on the basis of competition between the enzyme conjugate and the hormone in the sample for a limited number of binding sites on the antibody coated plate according to the method described by Wide et. al. (1976).

2. Histopathological techniques:

The collected samples of the testis and epididymis of each control and treated groups were fixed in 10% neutral buffered formalin solution, washed and routinely processed through paraffin embedding technique (dehydration in ascending alcohols, clearing, embedding in paraffin and blocking). Paraffin sections of 3-5 um thick were prepared then stained with hematoxylin and eosin according to the method described by Harries (1989) and subjected for light microscopy.

B. Experiments on pregnant female rats:

Pregnant female rats from control and treated rats were scarified on 19th day of gestation period by stunning against hard bench then opened to eviscerate the uterus of each female in each group to be morphologically examined by the method described by Hayes (1986) for:

- Number of foeti in each uterine horn.
- Livability of foeti in each uterine horn
- Weights of foeti in each uterine horn
- Size of foeti in each uterine horn.
- Number of corpora lute on each ovary corresponding to number of foeti in same horn of uterine horns.
- Presence of resorbed foeti in uterine horns.
- Presence of blood collection on uterine horns.
- External examination of foeti as absence of legs, fingers, head or tail.

5. Statistical analysis

The obtained data were statistically analyzed for variation among groups using SPSS computer program (SPSS, 2004). Data were presented as means plus or minus the standard error. The minimum level of significance was set at $P < 0.05$.

3. RESULTS:

3.1. Results of experiments on male goats:

a. Semen characteristics:

Both groups treated with Canobutin Sodium and Menbutone diethanolamine showed a significant decrease in sperm concentration, sperm progressive motility, Class A (Rapid progressive motility (>=25 μm/s at 37 °C) sperm % and Class B (Slow or sluggish progressive motility) sperm % and a high increase in Class C (Non progressive motility (<5 μm/s) sperm% and Class D (Immotile.) sperm % in comparison with the control group all-over the periods of experiment. Clanobutin sodium treated goats showed a significant decrease in normal sperm %, with a high increase in abnormal sperm % in comparison with the control group of male goats all-over the periods of the experiment (Table 1&2).

Normal sperm % and abnormal sperm % were not affected on the 2nd and 4th week of successive weekly menbutone diethanolamine treatment, but normal sperm% was decreased and abnormal sperm% was increased on 8th week of treatment. (Table 1&2).

Testicular and epididymal index weight were significantly decreased on both treated groups in comparison with the control group of male goats (Table 3).

b. ELISA results for hormonal levels:

After 2nd, 4th and 8th week of treatment, Canobutin Sodium and Menbutone diethanolamine induce a significant decrease on serum testosterone and a significant increase on FSH and LH levels (Table 4).

c. Histopathological studies:

Administration of clanobutin sodium on male goats for 8 successive weeks results on hyalinization of the luminal contents and sloughing of germinal epithelium in the lumen of the seminiferous tubules (Figure 1-B). While, Administration of menbutone diethanolamine on male male goats for 8 successive weeks results on buckling of the seminiferous tubules with congestion of interstitial testicular blood vessels. (Figure 1-C) in comparison with the control group of male goats (Figure 1-A).

Administration of clanobutin sodium on male goats for 8 successive weeks results on interstitial congestion of epididymal blood vessels and moderate numbers of mononuclear cell infiltration (Figure 2- B). While, administration of menbutone diethanolamine on male goats for 8 successive weeks results on interstitial congestion of epididymal blood vessels with vacuolation of few germinal epithelial cells (Figure 2-C) in comparison with the control group of male goats (Figure 2-A).

3.2. Results of experiments on pregnant female rats:

Number of foeti (Table 5), weights of each feti (Table 6), number of live feti (Table 7) on both uterine horns of pregnant female rats was significantly decreased on both clanobutin sodium and menbutone diethanolamine treated groups of rats in comparison with the control group. While, number of dead feti on both uterine horns of pregnant female rats was significantly increased on both clanobutin sodium and menbutone diethanolamine treated groups of rats in comparison with the control group (Table 8). Moreover, number of corpora lutea on both ovaries of pregnant female rats was non-significantly different on both clanobutin sodium and menbutone diethanolamine treated groups of rats in comparison with the control group (Table 9).

Table (1): The effect of intramuscular administration of clanobutin sodium and menbutone diethanolamine each alone on semen characteristics of male goats:

Parameter	periods	Control	Clanobutin sodium	Menbutone diethanolamine
Sperm concentration (sperm/ml)x 10^6	2nd week	A 1539.08±249.55	B 712.66±195.30	B 749.21±145.09
	4th week	A 1241.78±60.32	B 569.93±111.56	B 576.61±111.55
	8th week	A 1520.32±208.67	C 696.94±18.19	B 889.48±15.91
Progressive motility %	2nd week	A 80.07±1.22	B 56.95±7.16	B 54.14±4.21
	4th week	A 82.85±0.86	C 33.91±3.28	B 53.95±10.55
	8th week	A 83.62±2.24	C 26.01±2.25	B 77.00±4.58
Normal sperm %	2nd week	A 89.45±1.34	B 86.98±4.46	A 90.96±1.91
	4th week	A 93.00±1.25	B 80.72±7.87	A 92.12±3.22
	8th week	A 96.51±0.77	C 73.99±11.84	B 83.93±3.71
Abnormal sperm %	2nd week	B 10.55±1.34	A 13.02±4.46	B 9.04±1.91
	4th week	B 7.00±1.25	A 19.28±7.87	B 7.88±3.22
	8th week	C 3.49±0.77	A 26.01±11.84	B 16.07±3.71

*Means carrying different letters with the same raw are significantly different ($P<0.05$).

*Values are expressed as mean ± S.E *N= 5

Table (2): The effect of intramuscular administration of clanobutin sodium and menbutone diethanolamine each alone on motile sperm classification % of male goats

Parameter	Periods	Control	Clanobutin sodium	Menbutone diethanolamine
Class A %	2nd week	A	B	B
		39.87±2.22	23.31±2.43	23.03±5.04
	4th week	A	C	B
		35.60±1.30	15.76±1.14	26.29±4.53
	8th week	A	B	A
		27.44±1.11	13.27±1.37	27.90±0.75
Class B %	2nd week	A	B	C
.		40.21±2.50	33.64±5.41	28.11±9.62
	4th week	A	C	B
		47.25±1.11	17.99±2.42	27.66±7.04
	8th week	A	C	B
		56.18±3.17	12.73±1.40	49.10±4.55
Class C %	2nd week	B	A	C
		3.52±1.08	4.14±1.03	2.19±0.62
	4th week	C	A	B
		6.39±0.95	9.21±3.58	7.31±2.78
	8th week	B	C	A
		2.34±1.20	0.33±0.16	3.61±1.39
Class D %	2nd week	C	B	A
		16.41±1.20	38.91±6.36	46.67±14.58
	4th week	C	A	B
		10.76±1.30	58.27±7.28	38.74±10.85
	8th week	C	A	B
		14.03±1.12	73.66±2.18	19.39±3.95

*Means carrying different letters with the same raw are significantly different (P<0.05).
*Values are expressed as mean ± S.E *N= 5
Class A = Rapid progressive motility (>=25 µm/s at 37 °C) **Class B =** Slow or sluggish progressive motility
Class C = Non progressive motility (<5 µm/s) **Class D =** Immotile.

Table (3): The effect of intramuscular administration of clanobutin sodium and menbutone diethanolamine each alone on reproductive organs weight index of male goats:

Parameter	Control	Clanobutin sodium	Menbutone diethanolamine
Testis index weight (%)	A	B	B
	0.55±0.03	0.45±0.02	0.46±0.01
Epididymis index weight(%)	A	B	B
	0.10±0.01	0.07±0.01	0.07±0.01

*Means carrying different letters with the same raw are significantly different (P<0.05).
*Values are expressed as mean ± S.E., n= 5

Table (4): The effect of intramuscular administration of clanobutin sodium and menbutone diethanolamine each alone on hormonal parameters of male goats:

*Values are expressed as mean ± S.E., n= 5

Parameter	Periods	Control	Clanobutin sodium	Menbutone diethanolamine
Serum Testosterone (ng/ml.)	2nd week	A 1.50±0.17	B 1.30±0.10	B 1.22±0.11
	4th week	A 0.53±0.07	C 0.35±0.06	B 0.46±0.02
	8th week	A 0.57±0.07	C 0.45±0.02	B 0.49±0.02
Serum FSH (IU/L.)	2nd week	C 9.53±0.98	A 11.45±1.30	B 10.50±0.27
	4th week	C 7.53±0.78	B 8.42±0.31	A 9.18±0.82
	8th week	B 3.86±0.37	A 5.25±0.79	A 5.62±0.49
Serum LH (IU/L.)	2nd week	B 1.22±0.03	A 1.60±0.17	A 1.50±0.14
	4th week	B 1.46±0.09	A 1.70±0.23	A 1.82±0.18
	8th week	B 0.94±0.11	A 1.10±0.14	A 1.16±0.21

*Means carrying different letters with the same raw are significantly different (P<0.05)

Table (5). The effect of single daily i.m administration of clanobutin sodium and menbutone diethanolamine on the number of foeti of pregnant female rat:

No. of foeti in each horn	Control	Clanobutin sodium	Menbutone diethanolamine
Right uterine horn	A 4.80±0.37	B 3.50±0.56	B 3.33±0.80
Left uterine horn	A 4.20±0.73	B 3.33±1.11	B 3.33±0.76
Overall mean	**A 9.00±1.10**	**B 6.83±1.67**	**B 6.66±1.56**

*Means carrying different letters with the same raw are significantly different (P<0.05).

*Values are expressed as Means ± S.E. n=15

Table (6). The effect of single daily i.m. administration of clanobutin sodium and menbutone diethanolamine on the weight of foeti in each uterine horn of pregnant female rats.

weight of foeti in each horn	Control	Clanobutin sodium	Menbutone diethanolamine
Right uterine horn (g)	A 1.47±0.03	B 1.09±0.03	B 1.10±0.06
Left uterine horn (g)	A 1.37±0.02	B 0.89±0.03	B 0.90±0.06
Overall	A 1.42±0.02	B 0.99±0.03	B 1.00±0.06

*Means carrying different letters with the same raw are significantly different (P<0.05).

*Values are expressed as Means ± S.E. n=15

Table (7). The effect of single daily i.m. administration of clanobutin sodium and menbutone diethanolamine on the number of live foeti in each uterine horn of pregnant female rats.

No. of live foeti in each horn	Control	Clanobutin sodium	Menbutone diethanolamine
Right uterine horn	A	B	B
	4.80±0.37	1.66±0.55	1.66±0.66
Left uterine horn	A	B	B
	4.20±0.73	1.66±0.80	1.33±0.71
Overall mean	**A**	**B**	**B**
	9.00±1.1	**3.33±1.35**	**2.99±1.37**

*Means carrying different letters with the same raw are significantly different (P<0.05).

*Values are expressed as Means ± S.E. n=15

Table (9). The effect of single daily i.m. administration of clanobutin sodium and menbutone diethanolamine on number of dead foeti in each uterine horn of pregnant female rats.

No. of dead foeti in each horn	Control	Clanobutin sodium	Menbutone diethanolamine
Right uterine horn	B	A	A
	00±0.00	1.83±0.47	1.66±0.61
Left uterine horn	B	A	A
	00±0.00	1.50±0.50	2.00±0.85
Overall mean	**B**	**A**	**A**
	00±0.00	**3.33±0.97**	**3.66±1.46**

*Means carrying different letters with the same raw are significantly different (P<0.05).

Table (9). The effect of single daily i,m. administration of clanobutin sodium and menbutone diethanolamine on the number of corpora lutei of pregnant female rat.

No. of CL in each ovary	Control	Clanobutin sodium	Menbutone diethanolamine
Right ovary	A	A	A
	4.80±0.37	4.50±0.22	4.16±0.47
Left ovary	A	A	A
	4.20±0.73	4.50±0.76	4.50±0.22
Overall mean	**A**	**A**	**A**
	9.00±1.10	**9.00±0.98**	**8.66±0.69**

*Means carrying different letters with the same raw are significantly different (P<0.05).

*Values are expressed as Means± S.E. n=15

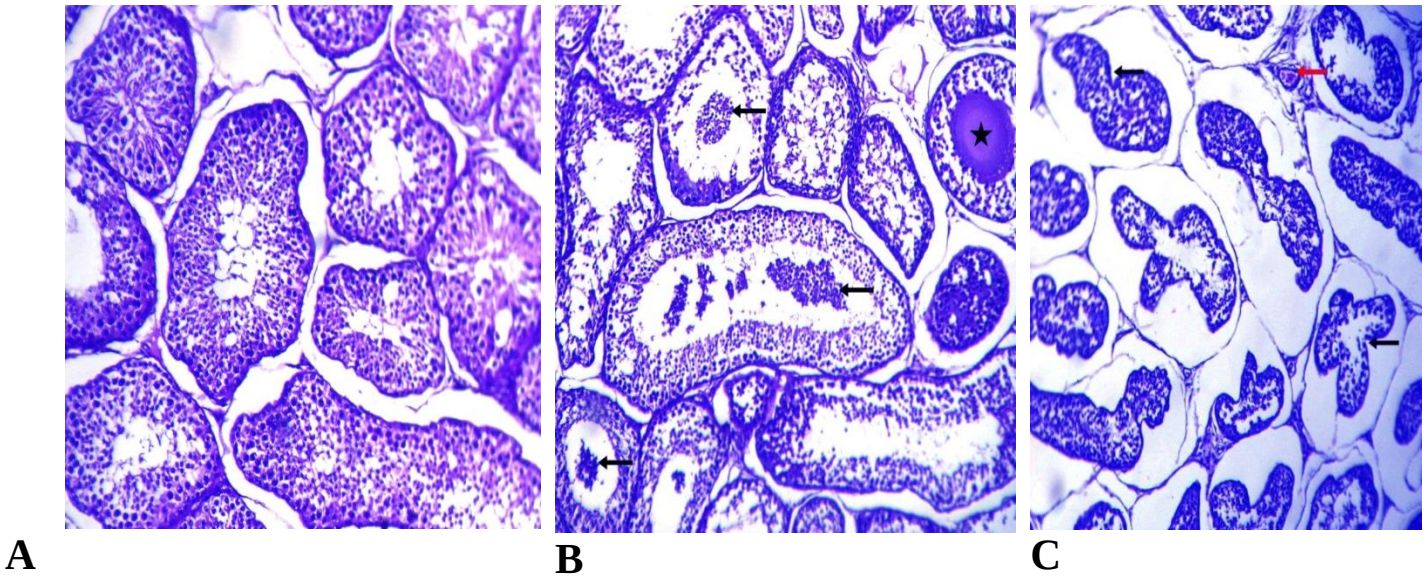

Fig.1: Seminiferous tubules of control mature male goat (A): showing normal histological structure of the testicular tissues. Seminiferous tubules of mature male goat treated with clanobutin sodium (B) showing hyalinization of the luminal contents (star) and sloughing of germinal epithelium in the lumen of the seminiferous tubules (arrows). Seminiferous tubules of mature male goat treated with menbutone diethanolamine (C) showing buckling (black arrows) and congestion of interstitial blood vessels (red arrow). Magnification was (X200).

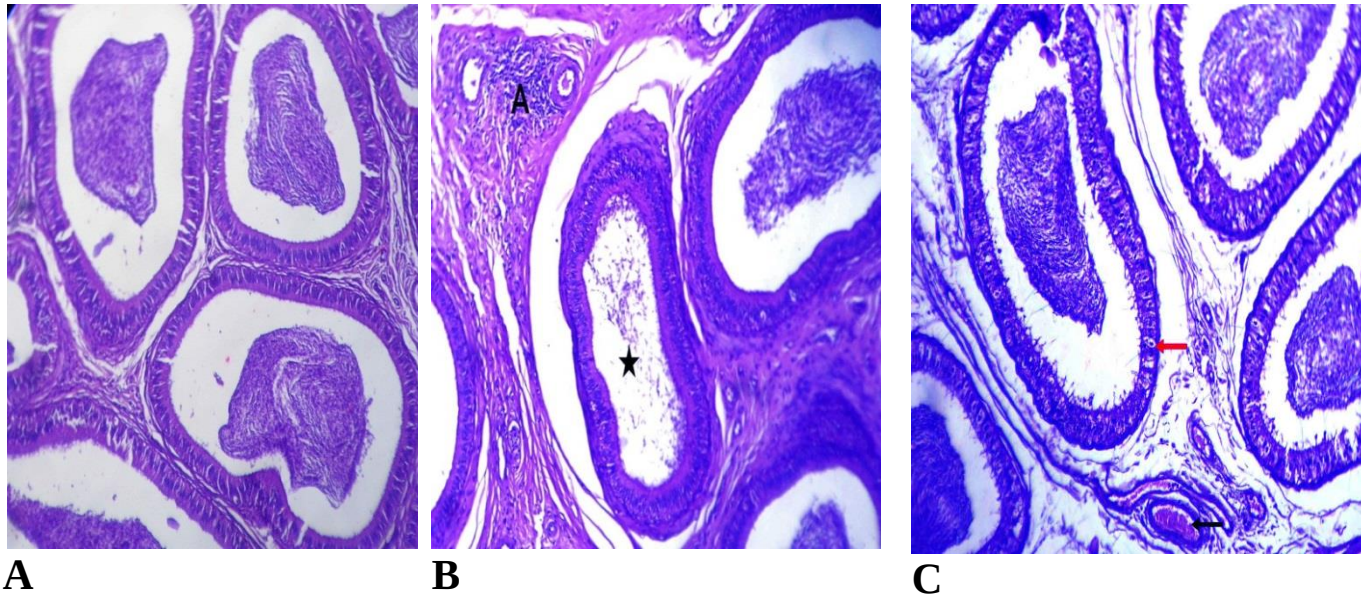

Fig. 2: Epididymis of mature male goat (control group) showing normal histological structure of the epididymal tissues. (A)., Epididymis of mature male goat treated with clanobutin sodium (B) showing interstitial congestion of blood vessels (star) and moderate numbers of mononuclear cell infiltration (A).; Epididymis of mature male goat treated with menbutone diethanolamine (C) showing interstitial congestion of blood vessels (black arrow) and vacuolation of few germinal epithelial cells (red arrow). Magnification was (X200).

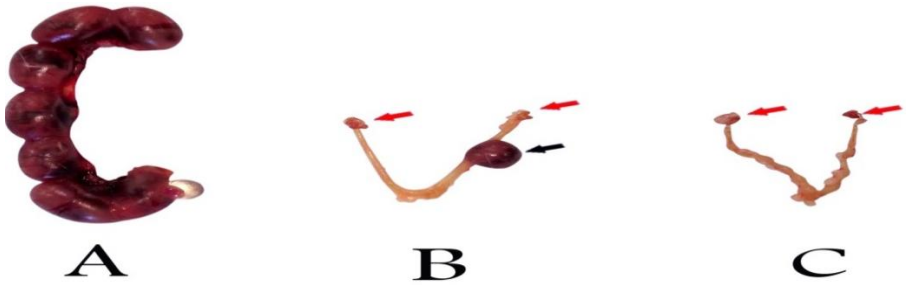

Figure 3. Uterus of female rat from the control group (A), uterus of clanobutin sodium treated female rat (B) showing a single stunted foeti on uterus of female rats (black arrow) and uterus of menbutone diethanolamine treated female rats (C) showing both uterine horns are devoid of foeti due to early embryonic death of all foeti. All ovaries carrying multiple corpra lutea (red arrows). All rats were scarified on day 19th of gestation period.

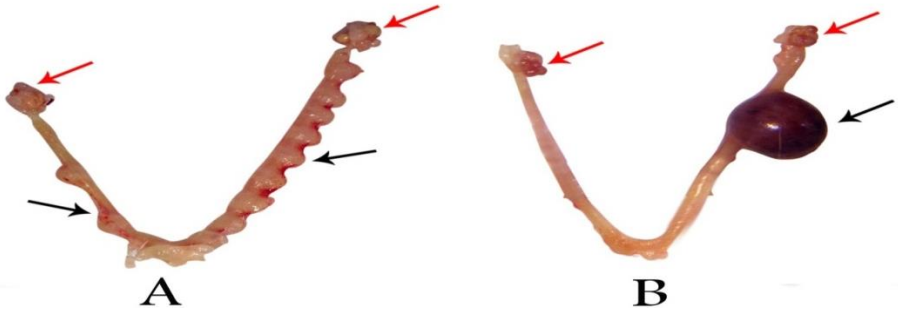

Figure 4. Uterus of menbutone diethanolamine treated female rat (A) showing Multiple corpora lutea on both ovaries of both uteri (red arrows) with multiple early dead foeti (black arrows), and uterus of clanobutin sodium treated female rat (B) showing Multiple corpora lutea on both ovaries (red arrows) with single stunted foeti (black arrow). Both of them were isolated on 19th day of gestation period.

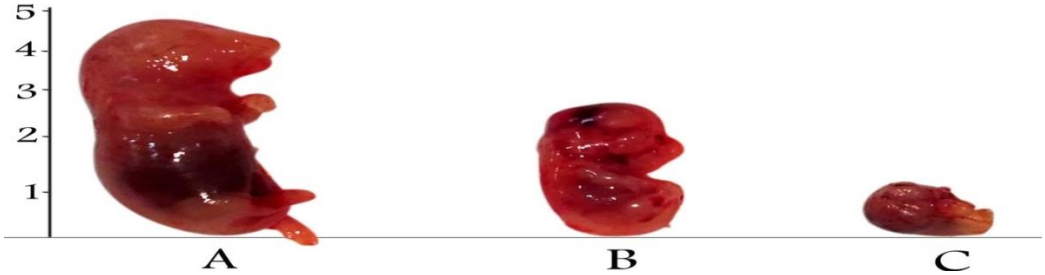

Figure 5. foetus of control group (A) with normal size of foeti in 19th day of gestation period, small stunted foetus of menbutone diethanolamine treated female rat (B) and deformed resorbed foetus of clanobutin sodium treated female rat (C). All of them were isolated on 19th day of gestation period.

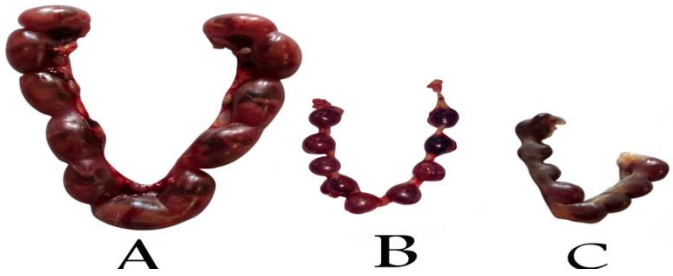

Figure 6. Uterus of female rat from the control group (A) showing normal sized foeti, uterus of clanobutin sodium treated female rat (B) showing multiple stunted dead foeti and uterus of menbutone diethanolamine treated female rat (C) showing multiple stunted dead foeti. All of them were sacrificed on day 19th of pregnancy period.

4. DISCUSSION

In the present study, the effect of clanobutin sodium and menbutone diethanolamine each alone on some male and female fertility parameters was investigated.

The present work showed that clanobutin sodium and menbutone diethanolamine induced a significant reduction on testicular and epididymal index weights with a highly significant decrease in sperm concentration, sperm progressive motility, normal sperm %, Class A sperm % and Class B sperm %, but there was a significant increase in abnormal sperm %, Class C sperm% and Class D sperm % in both treated group of male goats in comparison with the control group all-over the periods of the experiment.

These results clearly proven the deteriorating effect of clanobutin sodium and menbutone diethanolamine on male fertility that may be due to these drugs pass the blood testicular barrier resulting in very harmful effect on spermatogenic cells, this mechanism is confirmed by our histopatholgical findings on the testis and epididymis where, clanobutin sodium and menbutone diethanolamine administration induced various dangerous histopathological alterations (hyalinization of the luminal contents and sloughing of germinal epithelium in the lumen of the seminiferous tubules in both treatment) reducing livability and motility of performed stored spermatozoa.

These results agreed with Bedford (1975) and Orgebin-Crist et al. (1976), who mentioned that histopathological changes in testis and epididymis would change the medium necessary for sperm maturation and storage resulting in marked alteration in semen characters and male fertility.

The results are confirmed with our findings on the hormonal assay in which there were a significant decrease in testosterone level and a significant increase in FSH and LH levels in clanobutin sodium treated group and menbutone diethanolamine treated of male goats in comparison with the control group of male goats indicating hyper-gonadotropic hypo-gonadism or primary hypo-gonadism.

The reduction on serum total testosterone level may be a main cause on deteriorating effects of these drugs on male fertility. Alexander (1978) mentioned that the development and maintenance of sexual male organs functions and accessory sex organs and their secretion depends upon androgens secretion.

Hypogonadism is a lack of testosterone in males and can be of central (hypothalamic or pituitary) or testicular origin, or a combination of both. Hypogonadism in males with testicular failure due to genetic disorders, orchitis, trauma, radiation, chemotherapy, or undescended testes, is known as hypergonadotropic hypogonadism or primary hypogonadism. Hypogonadism in males with gonadotropin deficiency or dysfunction as a result of disease or damage to the hypothalamic pituitary axis is known as hypogonadotropic hypogonadism, central hypogonadism, or secondary hypogonadism. This might be due to tumor, trauma, radiation, or tuberculosis (Matsumoto, 2002).

Our results were in agreement with Christina (2004), who concluded that hypogonadism of testicular origin accompanied with decreased testosterone level and increase in FSH and LH levels. While, hypogonadism of hypothalimc-pitutary origin was accompanied with decreased testosterone level with decreased FSH and LH levels.

Moreover, Bretveld et al. (2007), reported that chemicals and pesticides may directly damage spermatozoa, alter Leydig cell function, or disrupt the endocrine function in any stage of hormonal regulation (hormonal synthesis, release, storage, transport and clearance; receptor recognition and binding; thyroid function and the central nervous system).

Regarding to the effect of these drugs on embryos of pregnant female rats, drugs administration was carried out at 5^{th} to 15^{th} day of pregnancy as this time was considered the most sensitive period of teratogensis and embryotoxicity

the results obtained in our work showed a significant decrease on the number of foeti in both uterine horns corresponding to the number of corpora lutae, weight of foeti, number of live foeti and increased number of dead abnormal or resorbed foeti on each horn in both treated groups of rats in comparison with the control group.

Teratogensis and embryotoxicity are highly specific processes which mean the capacity of chemicals for inducing birth defects or emryotoxic effects significantly increasing foetal structural or functional abnormalities at doses below those causing maternal toxicity (Hayes, 1982).

Also, Collins and Collins (1979) previously reported that the mechanism of action of most embryotoxins occurred through interference with nucleic acid replication/transcription, or RNA translation, deficiency of energy supply for metabolism of the organism by restricting the availability of substrate either directly or through the presence of analogs or antagonists of vitamins, essential amino

acids or through inhibition of the essential enzymes for organogenesis.

Moreover, Hayes (1982) reported that most of the unexpected side effects of drugs in adult animals are reversible but in foeti, they are irreversible and lead to clear either structural or functional abnormalities in newly born.

These deteriorating effects on the embryos of pregnant female rats may be attributed to passing the water soluble clanobutin sodium through the placental blood barrier resulting on more accumulation of the drug on the foetal body than the maternal body. Such accumulation could be enhanced by the very simplified structure of female rat's placenta affecting the growth, development, livability and vitality of the foeti on the uterine horns by acting as inhibitors of foetal membrane enzymes involved in embryonic nutrition (Tuchman-Duplessis, 1975).

The decreased percent of total number of foeti, number of live foeti and weight of foeti in both treated groups may be attributed to the fact that many chemicals may destroy the active cellular DNA and so reduce biosynthesis of essential components, like protein and energy sources (ATP and NAD/ NADP) and consequently the foeatl growth and livability (Haschek and Rousseaux, 1993).

Also, the increased number of resorbed foeti and early embryonic dead foeti in both treated groups of rats compared to control rats could be interpreted by conclusions of Hafez (1970) and McFeely (1993). They recorded that many chemicals may induce chromosomal abrasions; ring and sticky chromosomes, centromere alterations, hypoploidy and polyploidy causing early embryonic deaths which eventually lead to foetal resorption.

Another mechanism for embryotoxicity caused by these drugs administration may be due to the induction of free radicals production on the foetal body and surrounding uterine environment. This mechanism could be interpreted by conclusions of Naziroglu et al. (1999), who reported that the production of free radicals by the drugs may be involved in embryotoxicity as the ability of the foetus to clear the free radicals is very low, thereby exposing it to an increased oxidative load that cause foetal abnormalities and retarding their growth, livability and vitality.

5- CONCLSIONS

From the obtained results, we can conclude that clanobutin sodium and menbutone diethanolamine have harmful deteriorating effects on male and female fertility and therefore, both drugs must never be used in male animals intended for breeding or in pregnant female animals.

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