Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2016, Volume 4, Issue 4
ISSN: 2321–3647(online)

Histological effects of toxoplasmosis and its treatments on male and female rats

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ABSTRACT

The testis section of toxoplasma infected male rats showed vacular degeneration of spermatogonia and spermatocytes. The seminiferous tubules revealed sever degeneration, shrinkage, necrosis, hemorrhages with disappearance of epithelial lining of the majority of seminiferous tubules. They were also appeared separated with an irregular outlines and surrounded by fibrin. Seminiferous tubule lumen contained little amount of sperm with appearance of giant cells, polymorph nuclear leukocyte and exfoliated cellular debris. The epididymal sections of *Toxoplasma gondii* infected male rats treated by DMSO showed infiltration of mononuclear and multinucleated giant cell. Epididymal sections also showed hyperplasia of duct lining and contained toxoplasma cyst. Ovary sections of infected female rats treated by DMSO revealed morphological differences compared to the non infected group treated with DMSO, they showed decreased primary and secondary follicle with increased follicle atresia. Uterine section of infected female rats treated by DMSO showed hypertrophy of the endometrium and myometrium, polymorphic inflammatory infiltration, fewer glands, increased endometrial thickness with congestion of vessels. Pyrimethamine and sulphadiazine nor cause histological changed neither potentiate the histological effects of toxoplasmosis in infected and non infected male and female rats.

Keywords: *Toxoplama gondii*, infection, male, female, rats, histology, pyrimethamine, sulphadiazine

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Received 23 March 2016, Accepted 28 March 2016

Please cite this article as: Ali AS *et al*., Histological effects of toxoplasmosis and its treatments on male and female rats. American Journal of Pharmacy & Health Research 2016.

INTRODUCTION

Many studies correlated between reproductive performance and Toxoplasmosis[1-3]. *Toxoplasma gondii* deteriorated the male and female reproductive performance in many experimental animals [4-9]. Toxoplasmosis decreased hypothalamic, pituitary and gonadal secretion [1,3,8]. Furthermore, toxoplasmosis also caused profound adverse effect on human reproductive functions [10]. Acute toxoplasmosis infection caused hypogonadotrophic gonadal insufficiency in male patients regardless of the course of the disease [11]. The women with toxoplasmosis may complain spontaneous abortions, stillbirths, intrauterine growth retardation, preterm deliveries, or fetal anomalies [12-13].

T. gondii infection is of particular public health interest due to the ability of this parasite to cause infertility, therefore this study was designed to investigate the effect of toxoplasmosis on histology of male and female rats reproductive systems.

MATERIAL AND METHOD

The study was carried out on 42 males and 42 females rats (*Rattus norvegicus*) ranging in weight from 250 to 300g, all rats were housed in an air-conditioned animal room at an ambient temperature of 23 ±2 C and in a 12h lightl / 12h dark cycle. Half of the males and females were infected intraperitoneally with 1 X 10^7 tachyzoites of *T. gondii* intraperitoneally [14]. Infected groups were examined for documentation of the infection with the using of real-time PCR. Infected groups (21 males and 21 females) and non infected group (21 males and 21 females) were divided into 3 subgroups (7 each) and treated with dimethyl sulphoxide (DMSO), sulphadiazine 12.5 mg/kg or pyrimethamine 200 mg/kg. Sulphadiazine and pyrimethamine were given in DMSO as a single oral daily dose for 60 days in males and 2 estrus cycles in females. At the end of the treatment period, testis, epididymis, of males and ovary and uterus of females were fixed in formalin for histological examination [15].

RESULTS AND DISCUSSION

Testicular and epididymal sections:

The sections of the testis of non infected male rats treated with DMSO showed normal histological structures. The seminiferous tubules were intact with normal appearance of basement membrane, primary and secondary spermatocytes. The lumen was full with sperms, and with intact Sertoli cells. The seminiferous tubules appeared adjacent to each other with regular outlines. The interstitial tissues were intact. Also there were no histological changes in the non infected male rats treated with sulphadiazine or pyrimethamine.

The testis section of toxoplasma infected male rats showed vacular degeneration of spermatogonia and spermatocytes. The seminiferous tubules revealed sever degeneration, shrinkage, necrosis, hemorrhages with disappearance of epithelial lining of the majority of seminiferous tubules. They were also appeared separated with an irregular outlines and surrounded by fibrin. Seminiferous tubule lumen contained little amount of sperm with appearance of giant cells, polymorph nuclear leukocyte and exfoliated cellular debris. Sloughing of Leydig cells was also recorded.

The sections of the testis of infected male rats treated with sulphadiazine or pyrimethamine showed improvement of testicular structure. The section appeared with less or absent of vacular degeneration, necrosis and hemorrhages. The seminiferous tubules appeared with intact epithelial lining and the lumen full with sperms. The interstitial tissues also appeared intact with almost normal structure. It appeared that pyrimethamine was more effective than sulphadiazine in attenuation of the testicular histological changes associated with toxoplasmosis.

The epididymal sections of non infected male rats treated with DMSO, sulphadiazine, or pyrimethamine, showed that the epididymal duct was lined with an epithelium composed of principal and basal cells. The luminal diameter and the thickness of the peritubular smooth muscle increases from the proximal to the distal regions Few sperm were found in the initial segment, but a large mass of sperm aggregates were located in the cauda.

The epididymal sections of *Toxoplasma gondii* infected male rats treated by DMSO showed infiltration of mononuclear and multinucleated giant cell. Epididymal sections also showed hyperplasia of duct lining and contained toxoplasma cyst. Sperms appeared impacted in the lumen along with desquamated cellular debris. However, some epididymal ducts appeared atrophied with low amount of sperms.

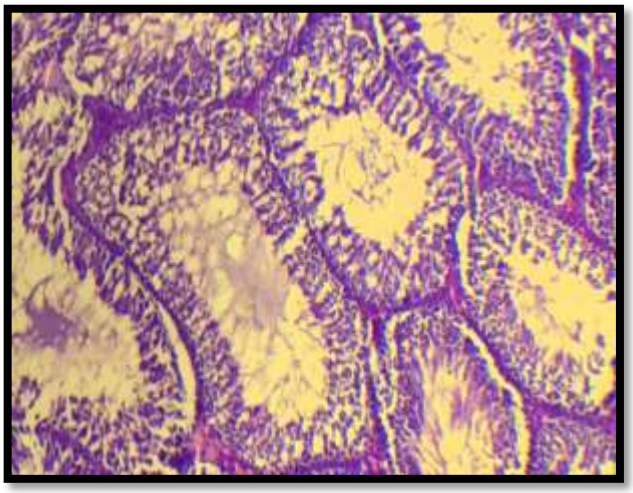
The epididymal sections of *Toxoplasma gondii* infected male rats treated with sulphadiazine, or pyrimethamine revealed hyperplasia of duct lining and slight degeneration. In the majority of the sections, the ducts appeared with normal diameter and epithelial lining, and the cavity was full with sperms.

Ovarian and uterine sections:

Ovary sections in non infected groups treated with DMSO, sulphadiazine, or pyrimethamine, showed normal histological pictures, the primary and secondary follicles and corpus luteum showed normal appearance. Ovary sections of infected female rats treated by DMSO revealed morphological differences compared to the non infected group treated with DMSO, they showed decreased primary and secondary follicle with increased follicle atresia, the atretic follicles were

characterized by degenerating oocytes, disorganized granulosa cell layers and folded zona pellucid. However, some of the primary follicles showed normal appearance. In the infected groups treated with sulphadiazine or pyrimethamine, the numbers of healthy primary and secondary follicles were increased with decreasing of the number of atretic follicles.

Uterine sections in non infected groups treated with DMSO, sulphadiazine, or pyrimethamine, showed normal histological pictures. The luminal and glandular epithelium were normal in appearance, normal thickness of endometrium and myometrium with low numbers of polymorphonuclear cells appeared within the lamina propria. Uterine section of infected female rats treated by DMSO showed hypertrophy of the endometrium and myometrium, polymorphic inflammatory infiltration, fewer glands, increased endometrial thickness with congestion of vessels in endometrium and myometrium. Uterine sections in infected groups treated with sulphadiazine, or pyrimethamine, also showed hypertrophy and increased endometrium and myometrium.



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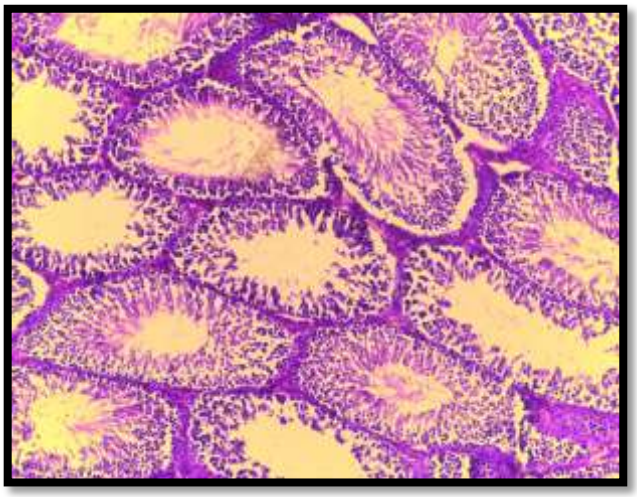

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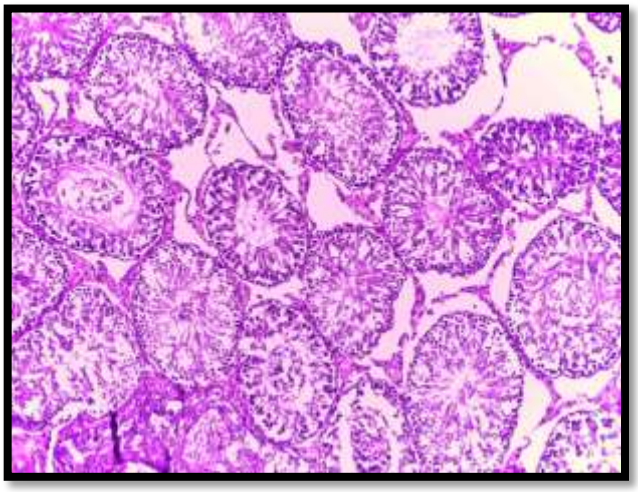

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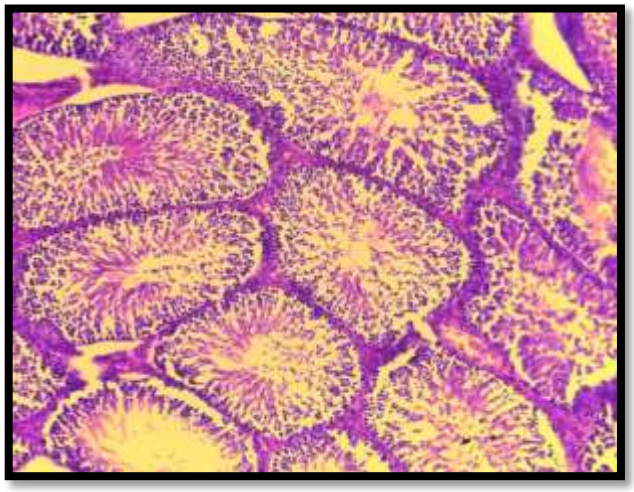

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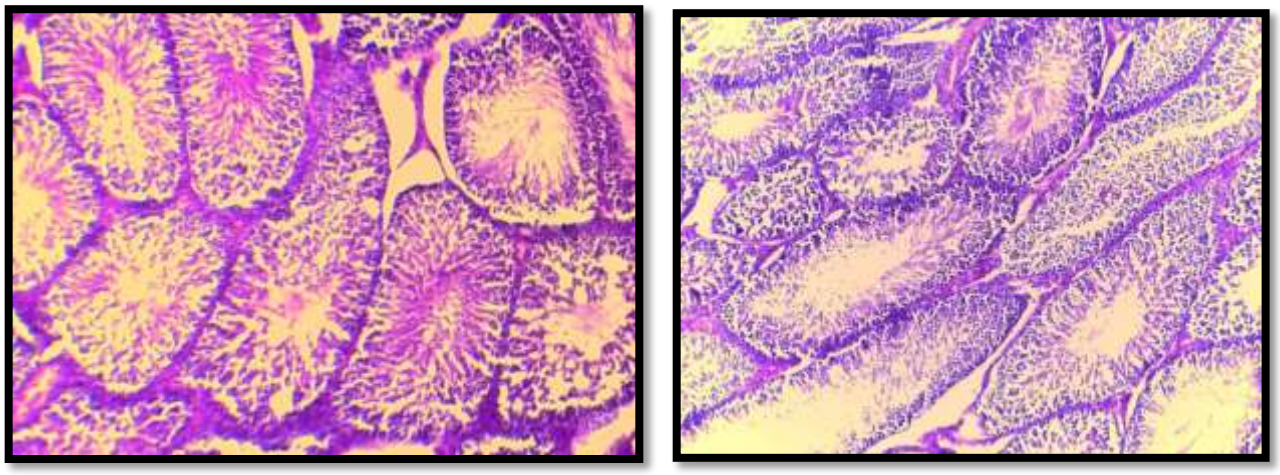
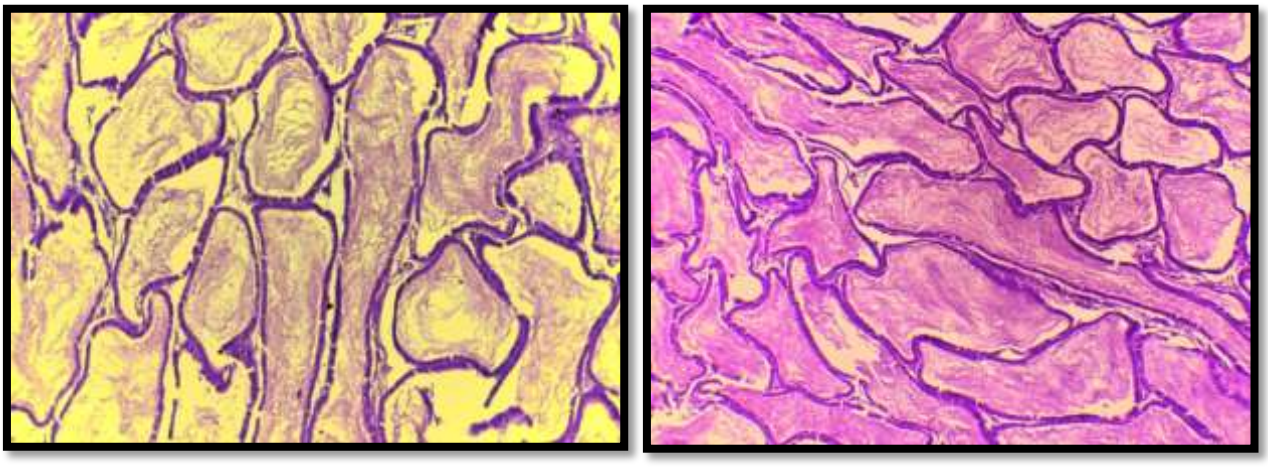


E **F**

Figure 1: testicular section of non infected male rats treated with DMSO

(A), pyrymethamine (B) and sulphadiazine (C) showed the normal histological structure. The testicular section of infected male rats treated with DMSO showed tubular degeneration, shrinkage, disappearance of epithelial lining of seminiferous tubules. They were also appeared separated and surrounded by fibrin. with appearance of giant cells, polymorph nuclear leukocyte and exfoliated cellular debris (D). The testicular section of infected male rats treated with pyrymethamine (E) and sulphadiazine (F) showed intact seminiferous tubules and lumen full with sperms. The interstitial tissues also appeared intact with almost normal structure (40X, H and E stain).



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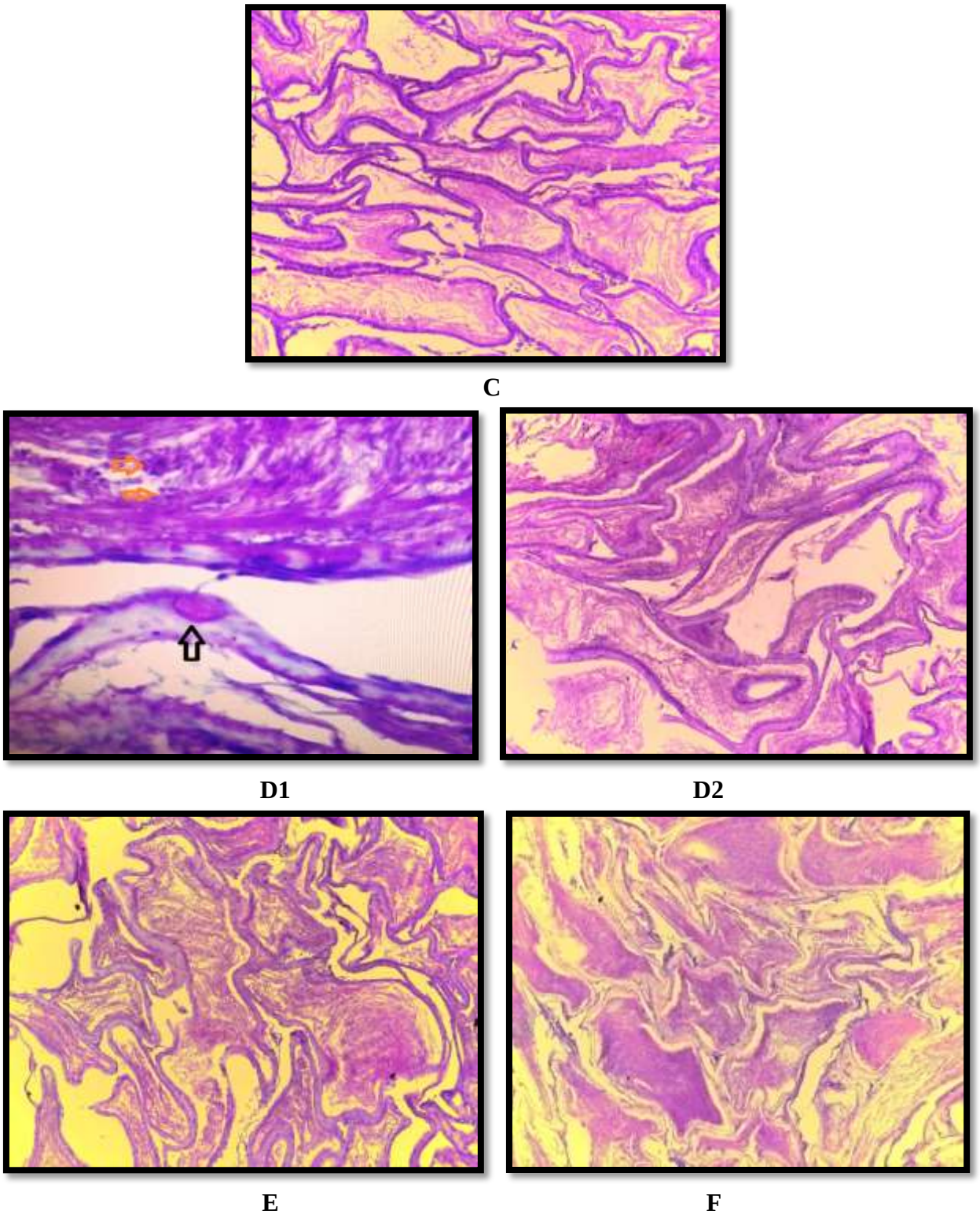


Figure 2: epididymal section of non infected male rats treated with DMSO

(A), pyrymethamine (B) and sulphadiazine (C) showed the normal histological structure. The epididymal section of infected male rats treated with DMSO showed hyperplasia of duct lining, sperms appeared impacted in the lumen (D1), with infiltration of inflamitarory cells (→) and appearance of toxoplasma cysts (↑) (D2). The epididymal section of infected male rats treated

with pyrymethamine (E) and sulphadiazine (F) also showed hyperplasia of duct lining (40X, H and E stain).

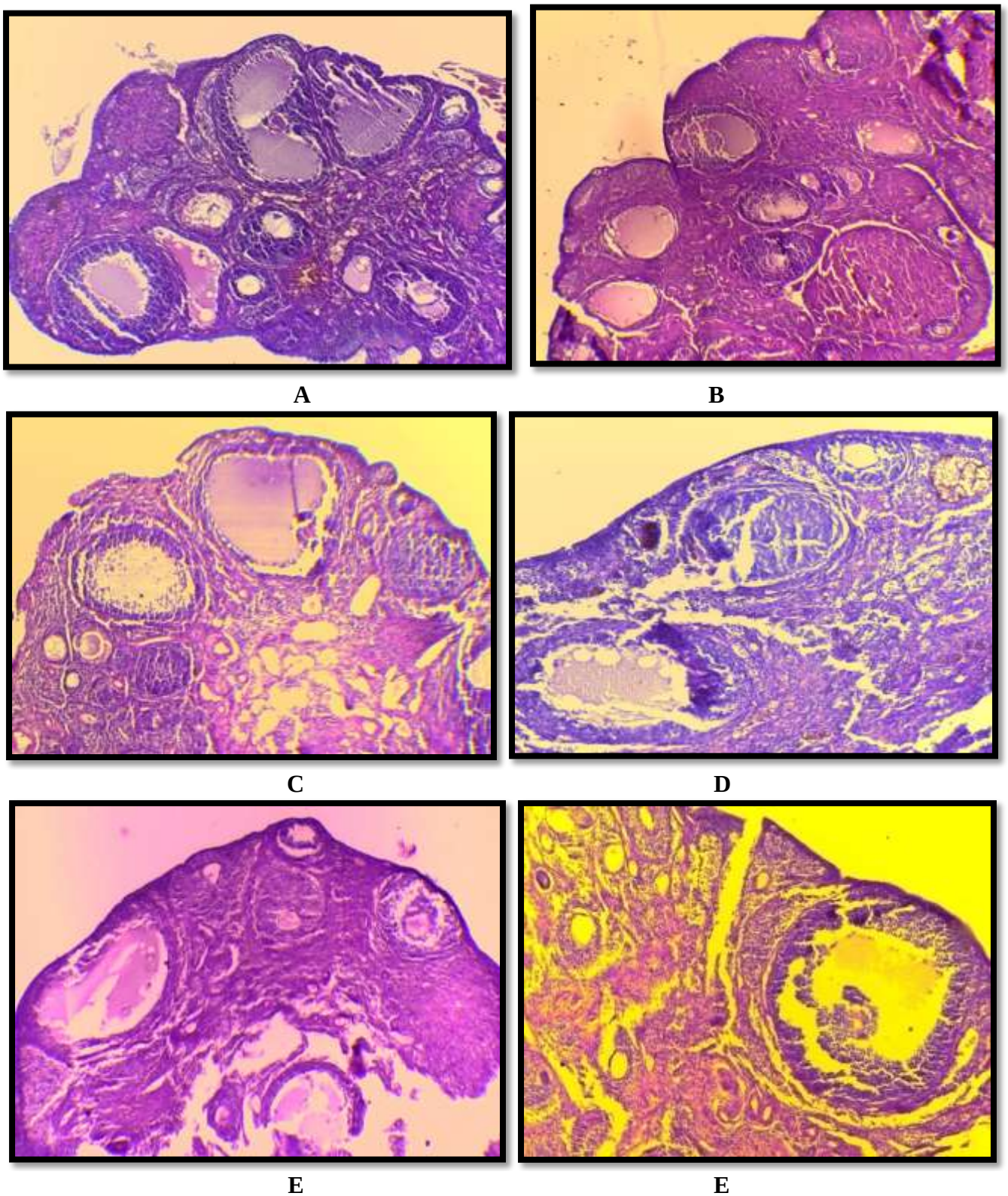
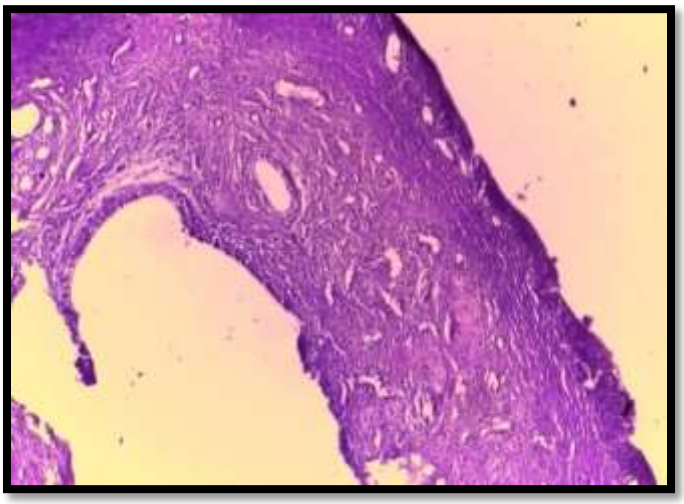


Figure 3: Ovarian section of non infected females treated by DMSO

(A), pyrimethamine (B) and sulphadiazine (C) showed the normal structure of the ovary. The histological section of the ovary of the infected female treated by DMSO showed decreased primary and secondary follicle with increased follicle atresia (D). The section of ovary of infected females treated by pyrimrthamine (E) and sulphadiazine (E) showed that the structure and number of ovarian follicles return to normal limit (40X, H and E stain).



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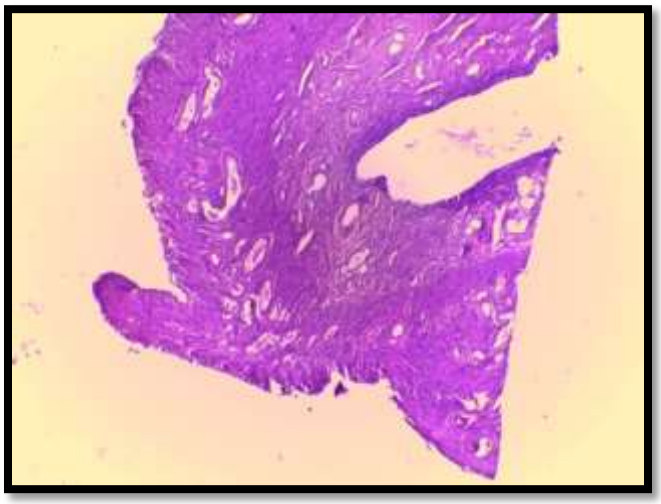

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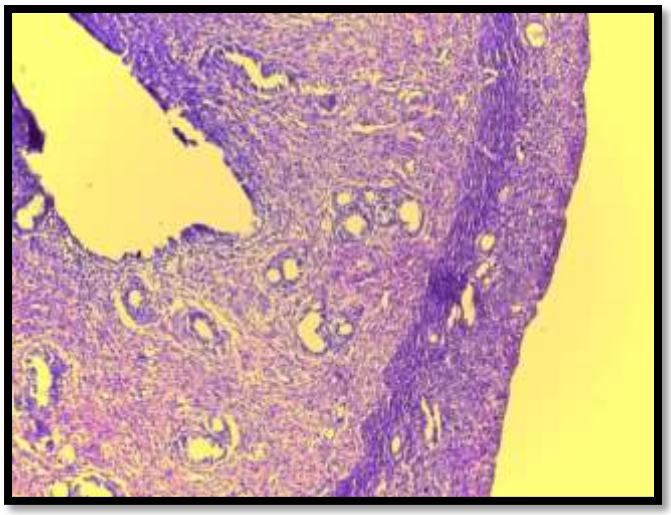

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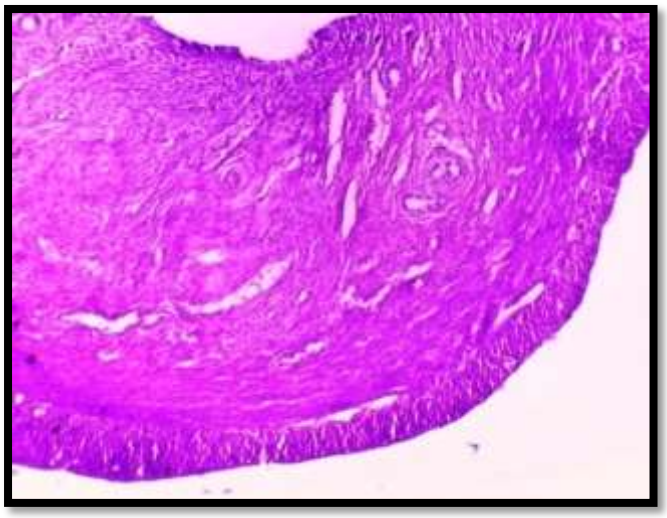

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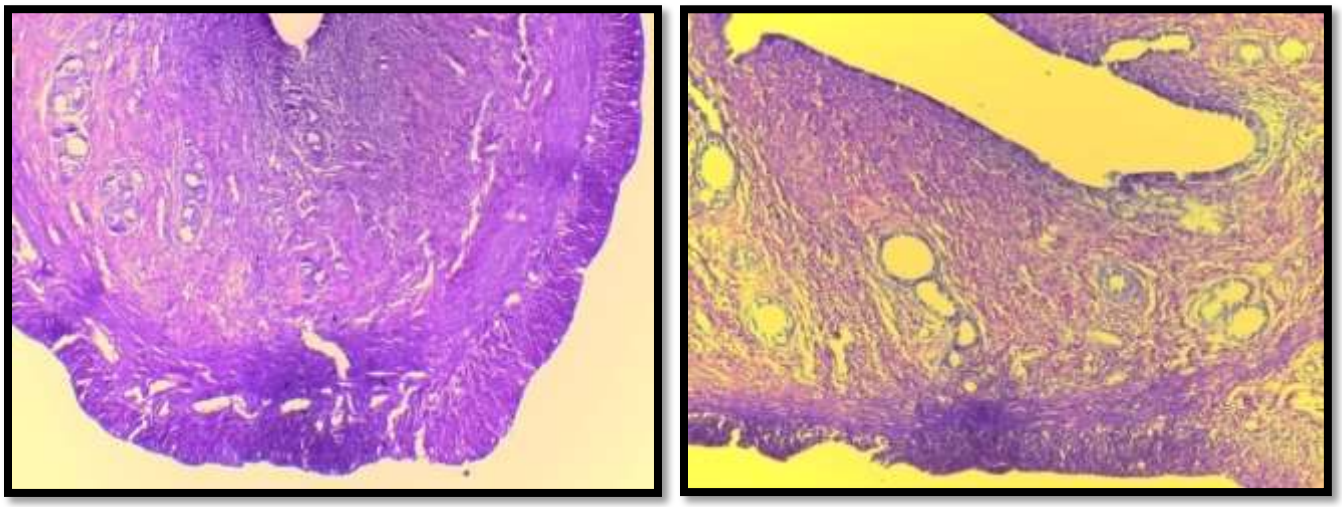

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Figure 4: Uterine section of non infected females treated by DMSO

(A), pyrimethamine (B) and sulphadiazine (C) showed the normal structure of the uterus. The histological section of the uterus of the infected female treated by DMSO showed hypertrophy of the endometrium and myometrium, polymorphic inflammatory infiltration and fewer glands (D). The uterine sections of infected females treated by pyrimrthamine (E) and sulphadiazine (E) also showed hypertrophy and increased thickness endometrium and myometrium (40X, H and E stain).

Our study revealed that the testis section of toxoplasma infected male rats showed vacular degeneration of spermatogonia and spermatocytes. The seminiferous tubules revealed sever degeneration, shrinkage, necrosis, hemorrhages with disappearance of epithelial lining of the majority of seminiferous tubules. They were also appeared separated with an irregular outlines and surrounded by fibrin. Seminiferous tubule lumen contained little amount of sperm with appearance of giant cells, polymorph nuclear leukocyte and exfoliated cellular debris. Sloughing of Leydig cells was also recorded. Furthermore, the epididymal sections of *Toxoplasma gondii* infected DMSO-treated male rats showed decreasing of the epithelial cell high and ducts diameters with infiltration of mononuclear and multinucleated giant cell. Epididymal sections also showed hyperplasia of duct lining, sperms appeared impacted in the lumen along with desquamated cellular debris. The histological changes found in testis and epididymis in this study were also recorded by Arantes *et al*., the histopathological study of the reproductive system (testicles and epididymis) of male dogs experimentally infected with *Toxoplasma gondii* showed mononuclear inflammatory infiltrate in the epididymis, moderate cellular edema, hydropic degeneration and moderate interstitial fibrosis in seminiferous tubules[16.]

Lopes *et al*. investigated the reproductive histology in small male ruminants after *Toxoplasma gondii* infection. They noted diffuse testicular degeneration associated with calcification foci and a multifocal mononuclear interstitial inflammatory infiltrate; and a mononuclear interstitial infiltrate and focal necrotic areas of the muscle fibers surrounding the seminal vesicles[17].

The histological changes in the ovary and uterus in *Toxoplasma gondii* infected female rats were also recorded previously. Eslamirad *et al*., mentioned that the ovaries of *Toxoplasma gondii* infected pregnant mice showed gross morphological differences compared to the control groups. Primary follicles, secondary follicle and corpus luteum were significantly decreased in the infected females [18]. However, Fux et al., recorded accentuated hypertrophy of the endometrium and myometrium and a reduction in folliculogenesis and formation of corpora lutea in the ovaries of *Toxoplasma gondii* infected mice [19].

The histological changes could be attributed to the declined pituitary secretion and decreasing of its influence on male and female reproductive organs in the infected animals. Toxoplasmosis significantly decreased FSH and LH in both males and females, with a subsequent decline in the level of testosterone in males and estrogen and progesterone in females. Toxoplasmosis increased interleukin-1b (IL-1b) levels. The levels of IL-1b correlated significantly in a negative manner with FSH, LH, free testosterone (FT), total testosterone (TT) in all patients with acute toxoplasmosis[15]. Interleukin-1b suppressed the hypothalamic- pituitary- gonadal (HPG) axis, directly or indirectly through increased corticotrophin-releasing hormone (CRH) and/or cortisol [20]. Interleukin-1b released peripherally in response to the parasite, reached the hypothalamus and initiated a sequence of events that inhibited the pulsatile release of gonadotropin-releasing hormone (GnRH), leading to the subsequent impairment of the pituitary-gonads axis[6].

Our study also revealed that sulphadiazine and pyrimethamine didn't induce testicular, epididymal, ovarian and uterine histological changes in non infected males and females. The same results were recorded previously, Awoniyi *et al*., mentioned that pyrimethamine 100 mg/kg/day did not alter the testicular and epididymal structure. Histological picture of the testis of the treated male was indistinguishable from controls[21].

Many studies revealed that both drugs were free from endocrine effect, they didn't affected the hypothalamic – pituitary gonads axis both in males and females[21-22], and didn't affected the relative weights of sexual and secondary sexual organ, semen quality and sexual performance, which could be explain the normal testicular, epididymal, ovarian and uterine histological structures of male and female rats treated by pyrimethamine and sulphadiazine in this study.

Furthermore, both sulphadiazine and pyrimethamine attenuated the histological changes associated with toxoplasma infection in both males and females. The attenuation of histological changes in infected male and female rats treated by sulphadiazine or pyrimethamine could be attributed to the therapeutic effects of these drugs in toxoplasmosis[23-24].

CONCLUSION

Toxoplasmosis deteriorate the normal histological structure of sexual organs of both male and female rats. Pyrimethamine and sulphadiazine nor cause histological changed neither potentiate the histological effects of toxoplasmosis in infected and non infected male and female rats.

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