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RESEARCH ARTICLE

Electron micrograph studies on the effects of fluoxetine in depression-induced adult female rat ovaries

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Abstract

Background: Fluoxetine is a common drug in the treatment of major depressive disorders. However, its effects on the ovarian tissues are less explored. The objective of this study, therefore, is to examine the ultrastructural changes in the ovaries of depressed rats undergoing treatment with fluoxetine by electron micrograph (EM) analysis. Biochemical assays will indicate the functional aspects of the ovaries. **Methods/Statistical analysis:** The female Wistar rats selected for the study were given doses of reserpine drug to induce depression. Following this, treatment with fluoxetine at 10mg/kg and 20mg/kg was given. The blood samples were collected by retro-orbital method and used for the biochemical assays. Further, the analysis of the data was done by one-way ANOVA. The animals were sacrificed, ovaries isolated and processed for electron microscopy. The EM was observed and interpreted. **Findings:** EM studies on the ovarian tissues of depression-induced female rats undergoing treatment with 20mg/kg fluoxetine show the presence of an inconspicuous nucleus, several hydrated mitochondria, glycogen granules, fibroid encompassing the ovarian follicle and heavy invasion of stereo cilia. The biochemical assays also indicate a highly significant decrease in the ovarian hormones in this group at P=0.05(5%). **Applications:** The results are indicative of the detrimental effects of fluoxetine at 20mg/kg on the rat ovaries. Fluoxetine therapy for the treatment of depression in females should be in consideration with its influence on ovaries and ovarian hormones.

Keywords: Fluoxetine; electron micrographs; rat ovary

1 Introduction

Fluoxetine, approved for the treatment of depression, is a highly effective Selective Serotonin Reuptake Inhibitor (SSRI) in vitro and in vivo[(1)]. In clinical trials of antidepressants, the placebo effects with fluoxetine were found to be high in the range of 30% for subjects diagnosed with major depression[(2)]. In addition to the less aversive side effects and comparable efficacy, SSRIs in particular fluoxetine, has a wider therapeutic index[(3–6)]. The most benefits from fluoxetine tend to occur in people who have moderately severe illness[(5)].

Fluoxetine is beneficial in the treatment of depression, however, its effects on the female ovaries is to be clearly understood. It is observed that in rats, fluoxetine and norfluoxetine cross the placenta and distribute within the foetus during the periods of organogenesis and post organogenesis [7]. Fluoxetine at high concentrations were found to inhibit the contraction induced by potassium ion on the isolated rat uterus preparation [8]. Other studies reveal that there is a reduced libido in adult rats chronically exposed to fluoxetine [9]. Fluoxetine was also found to reduce the aggressive behavior displayed during the diestrus phase by normally cycling rats [10]. In addition to these behavioral and physiological responses to fluoxetine treatment, histological alterations in the reproductive organs are a possibility. Studies on the histological changes in rat ovaries in response to several chemicals were done using light and electron microscopy [11–15]. However, EM studies on ultrastructural changes of ovaries of depressed rats undergoing treatment with fluoxetine have not been worked out so far.

The ultrastructural changes in turn may affect the secretion of ovarian hormones. Research findings indicate that females and males use different hormonal and neural mechanisms to respond to the same emotional event [16]. The critical role of ovarian hormones in the behavioral, inflammatory and cardiovascular susceptibility to social stress in female rats has been noted [17]. It is also observed that the estrogen can pass the brain-blood barrier and bind to cytoplasm estrogen receptor (ER)-α and ER-β in different areas of the limbic system of the brain. During conditions of stress, estrogen can thus modulate the behavioral and neurobiological response depending on the concentrations of estrogen [18]. Estrogen replacement retrieved OVX (ovariectomy)-induced nociceptive hypersensitivity and depressive-like behaviors [19]. Estrogen E_2 and DHT (dihydrotestosterone) have some anti-depressant effects but the modest PNS (prenatal stress) may alter E_2's ability to alleviate some depressive behavior in female and not male rats [20]. These studies suggest that levels of estrogen and progesterone play an important role in major depressive disorders.

The current study investigates the dose effects of fluoxetine at 10mg/kg and 20mg/kg on the ultra-structure of ovaries and ovarian hormones in depression-induced female rats.

2 Materials and Methods

2.1 Animal treatment

Animal models of depression include the Flinders Sensitive Line (FSL) rat, the Wistar Kyoto (WKY) rat, the Fawn-Hooded (FH) rat and the Learned Helpless (LH) rat [21]. Female Wistar rats weighing between 100-150 kg initially (i.e., before leading them to develop depression clinically) were selected. The animals were obtained from Bharat Serum and Vaccines Ltd., Thane and were housed in the Animal House, R. Jhunjhunwala College, Ghatkopar, CPCSEA registration number 525/02/.a. The animals were acclimatized in the laboratory for about a week and were treated and cared for in accordance with Organization for Economic and Community Development (OECD) guidelines.

The animals were housed individually in polyurethane cages with wire mesh tops and rice husk bedding which was changed every day. The temperature in the experimental animal room was maintained at 22°C ($\pm$ 3°C). The humidity was around 50-60%. Artificial lighting with a sequence of 12 hours light and 12 hours dark was maintained. For feeding, conventional rodent laboratory diets were used and water was provided ad-libitum in bottles. Commercially available rat feed was supplied by 'Amrut' laboratory animal feed.

2.1.1 Experimental design

The female Wistar rats were sorted into four groups of 5 each. The drugs under study were dissolved in distilled water and all doses were given intraperitoneally in morning hours. Reserpine puriss (LOBA Chemicals) was injected for inducing depression [22–26]. Fluoxetine (Prozac) was used as an antidepressant for treatment.

a) Animal grouping and dosage

Group I (Control): Sterile distilled water 2cc/kg was administered for 21successive days.

Group II (Depression induced): Sterile distilled water 2cc/kg was administered for 21 successive days. Reserpine dose of 3mg/kg body weight was given for 7 successive days.

Group III (Depression induced + Treated): Reserpine 3mg/kg body weight was given for 7 successive days. Fluoxetine (10 mg/kg body weight) was administered for 21 successive days.

Group IV (Depression induced + Treated): Reserpine 3mg/kg body weight was given for 7 successive days. Fluoxetine (20 mg/kg body weight) was administered for 21 successive days.

b) Sample collection

At the end of each treatment blood samples were collected by retro-orbital method for biochemical assays. Animals were sacrificed after ether anesthesia by cervical dislocation, dissected, examined for any visible alterations in anatomy. Ovaries were

quickly removed and processed for Electron microscopy.

c) Biochemical assays

Serum estradiol and progesterone hormone was detected by radioimmunoassay.

d) Electron microscopy

Processing of tissue was done for routine electron microscopy. Fixation was done with 1% osmium tetrasulphate in sodium cacodylate buffer, followed by alcoholic degradation. Embedding of tissue blocks were done in BEEM capsules with fresh araldite 'B' solution. Ultrathin sections or 'thin sections' 600-900 A^0 were cut on a Leica ultra cutter R, with glass knives, prepared on a Leica (knife make) EMKMR2. Sections were collected on 300 mesh copper grids and observed on JEOL-1010 electron microscope.

3 Results and Discussions

3.1 Biochemical assays

Data obtained from experimental studies with each independent variable in four groups were analysed by One-way Analysis of Variance (ANOVA). The results obtained were statistically significant at P=0.05 (5%) (Table 1).

Table 1. Biochemical parameters analysed in different groups

Parameters	Group I	Group II	Group III	Group IV
Estradiol ng/ml (Serum)	34.0±0.55	19.12±0.08	29.1±0.07	16.12±0.08
Progesterone ng/ml (Serum)	24.1± 0.07	21.06 ± 0.05	22.14 ± 0.08	9.08 ± 0.08

3.2 Electron Micrograph (EM) studies

Group I: EM of rat ovary of control shows distinct irregularly shaped Nucleus (N). Intact mitochondria (M) with distinct cristae and dense matrix are visible. Some of the degenerating mitochondria (DM) are also observed (Figure 1). There is the presence of autophagic vacuole (AV) and Golgi bodies (G).

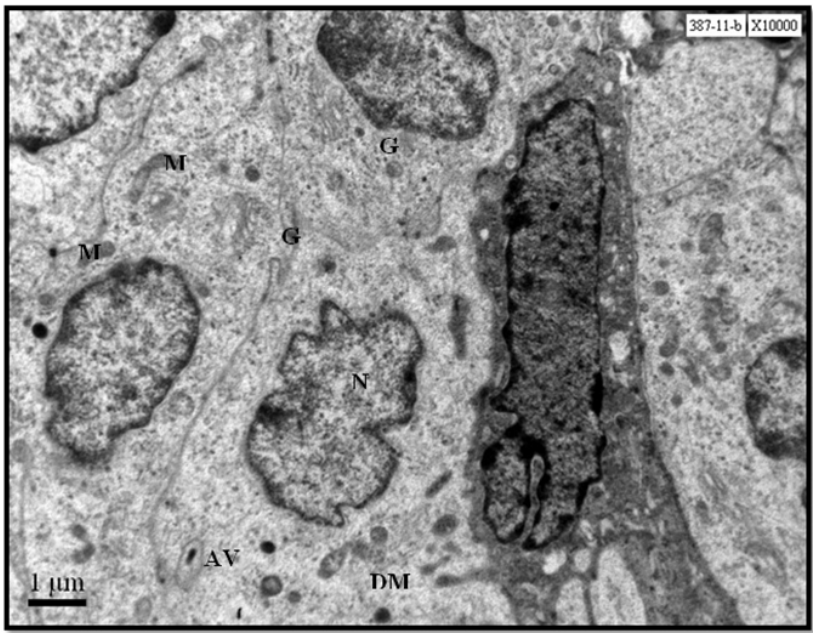

Fig 1. EM of rat ovary of control exhibiting distinct irregularly shaped Nucleus (N)

Group I: EM of rat ovary of control shows distinct Graafian follicle (GF) and ovarian follicle (F) under developmental stages (Figure 2). Also, note the peripheral stromal cells (S).

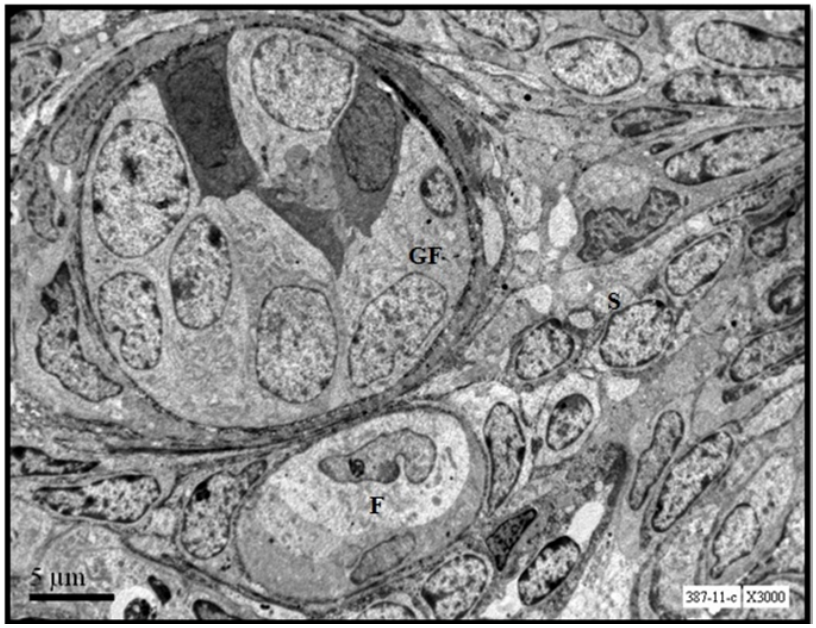

Fig 2. EM of rat ovary of control exhibiting distinct Graafian follicle (GF) and ovarian follicle (F) under developmental stages

Group II: EM of rat ovary treated with reserpine shows distinct nucleus (N) with the nucleolus (Nu). Note the polyribosome chains in the ovum (P). Several free ribosomes and polyribosomes give a granulated appearance to the cytoplasm (Figure 3). A stray appearance of crystalloids (C) is noticed. Ovum is with a thick membrane (M).

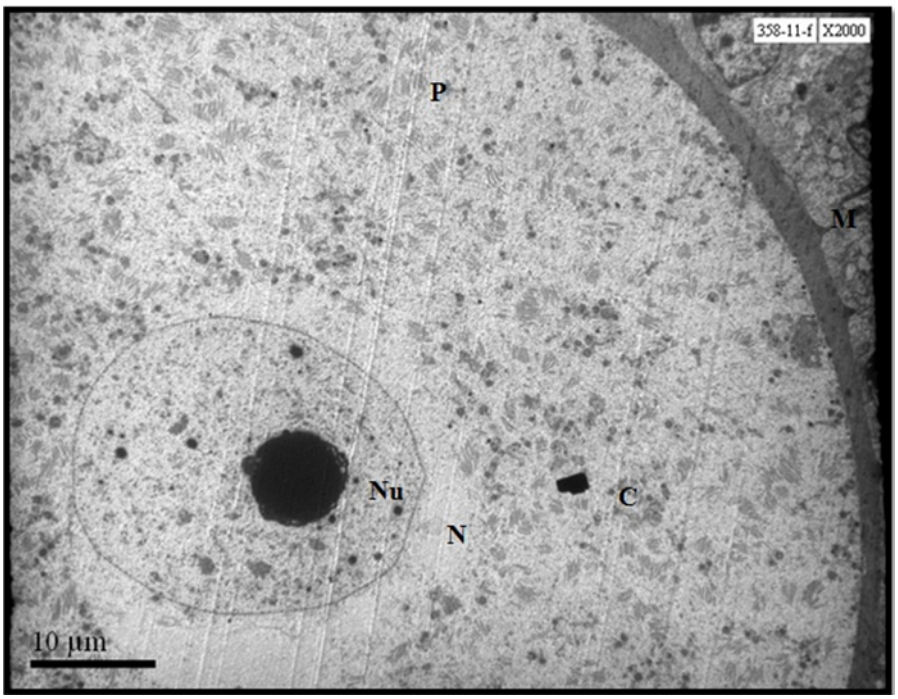

Fig 3. EM of rat ovary treated with reserpine shows distinct nucleus (N) with the nucleolus (Nu).

Group II: EM of rat ovary treated with reserpine shows Graafian follicle with distinct nuclei (N). The number of mitochondria (M) is more indicating a higher synthesis of ATP. Vacuole (V) and primary lysosomes (Ly) are seen (Figure 4).

Note the presence of a dense body (→).

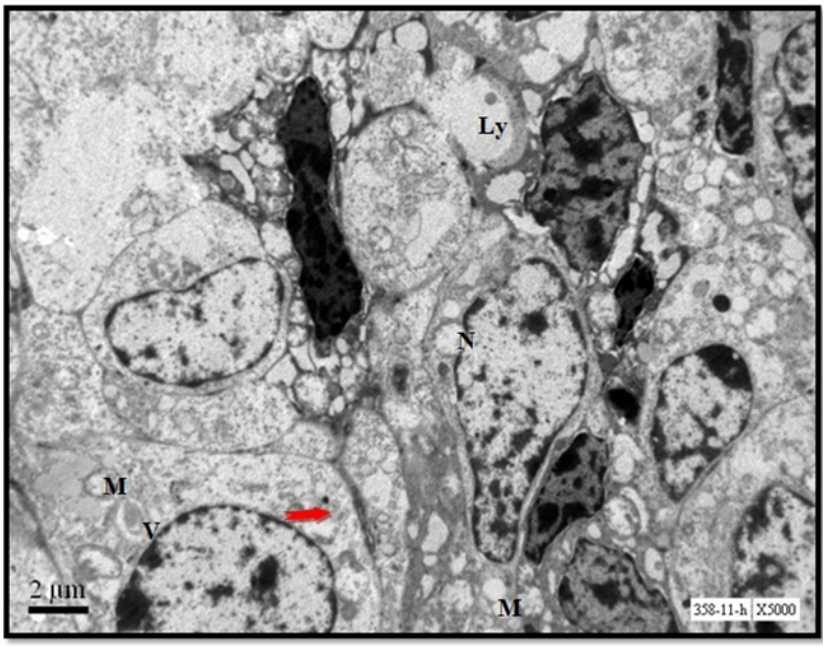

Fig 4. EM of rat ovarytreated with reserpine exhibiting Graafian follicle with distinct nuclei (N)

Group III: EM of rat ovary treated with fluoxetine 10mg/kg shows secretory granules (SG) which are polymorphic. Note the primary ovarian follicle undergoing degeneration (F). Several hydrated mitochondria (M) with loss of cristae are also observed (Figure 5).

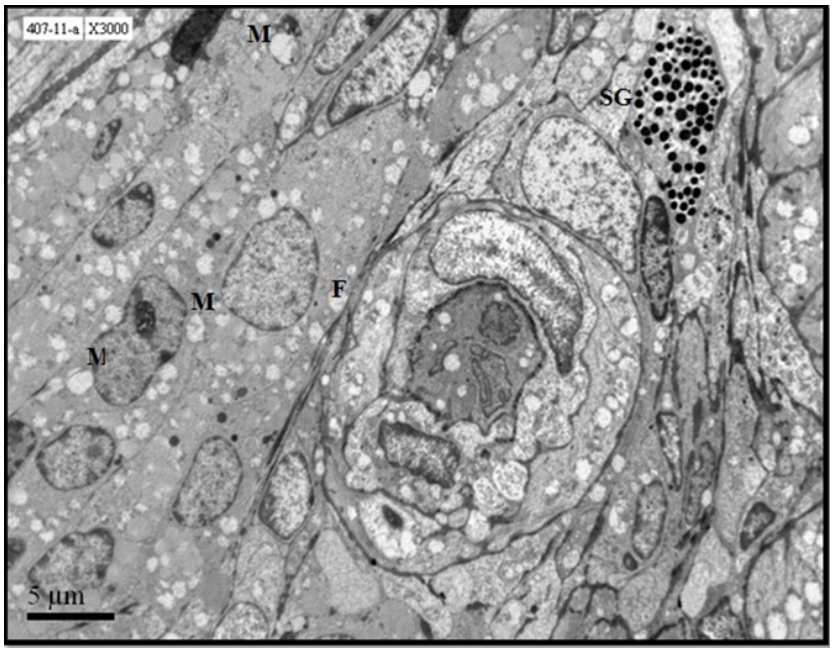

Fig 5. EM of rat ovary treated with fluoxetine 10mg/kg exhibiting secretary granules (SG)

Group III: EM of rat ovary treated with fluoxetine 10mg/kg with distinct mitochondria (M) and nucleus (N). Note the presence of Juxta nuclear Golgi region (G) in the follicle (F). There is a presence of autophagic vesicle (AV). Membrane blebbing

(→) are seen at several places indicating cell-cell interaction (Figure 6).

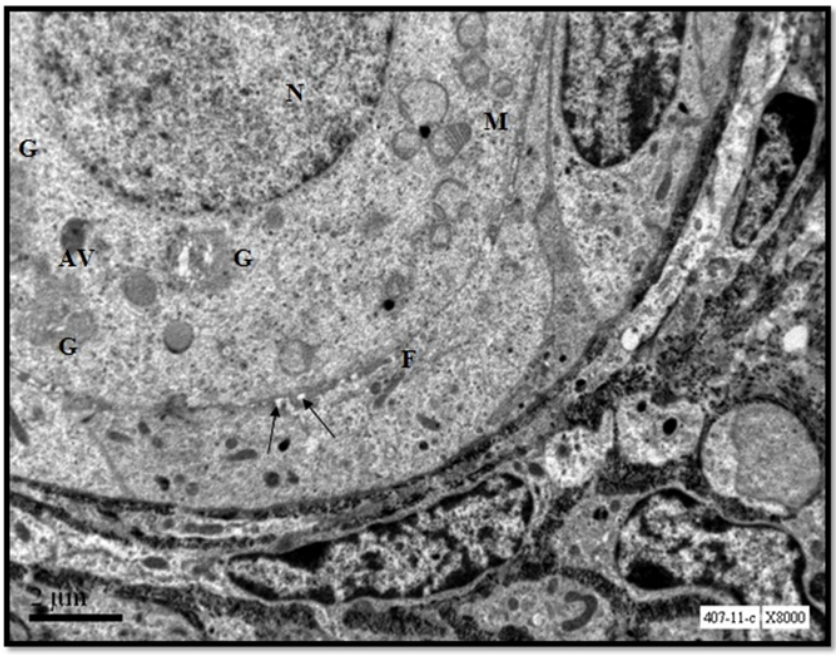

Fig 6. EM of rat ovary treated with fluoxetine 10mg/kg with distinct mitochondria (M) and nucleus (N)

Group IV: EM of rat ovary treated with fluoxetine 20mg/kg shows ovarian follicle completely degenerated with fibroid encompassing the follicle. Note the invasion of the sterocilia (SC) into the stroma of the follicle. The characteristic structure of ovarian follicle cell is disappearing (Figure 7).

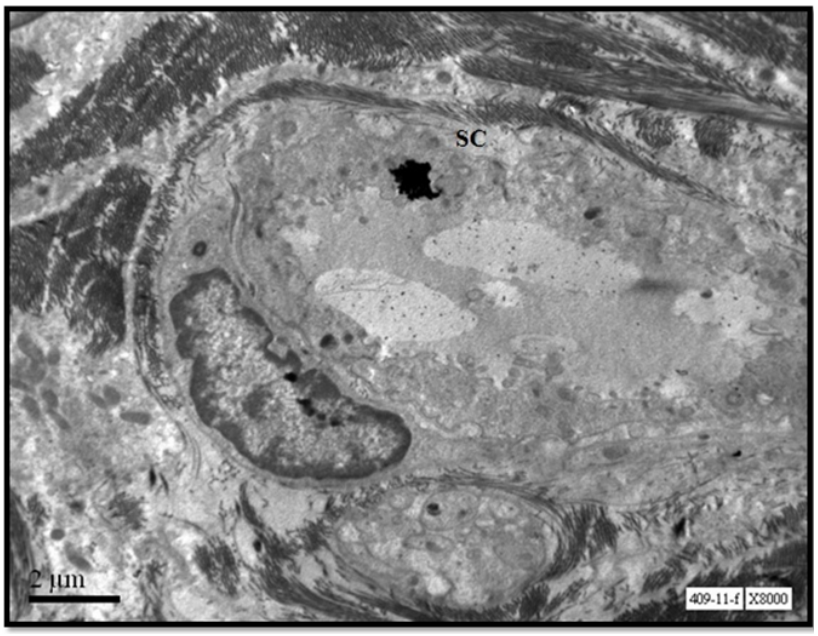

Fig 7. EM of rat ovary treated with fluoxetine 20mg/kg shows ovarian follicle completely degenerated

Group IV: EM of rat ovary treated with fluoxetine 20mg/kg with inconspicuous nucleus (N). Several hydrated mitochondria (M) and dense bodies (→) are also seen. Observe the myelin fibres (MF) (Figure 8).

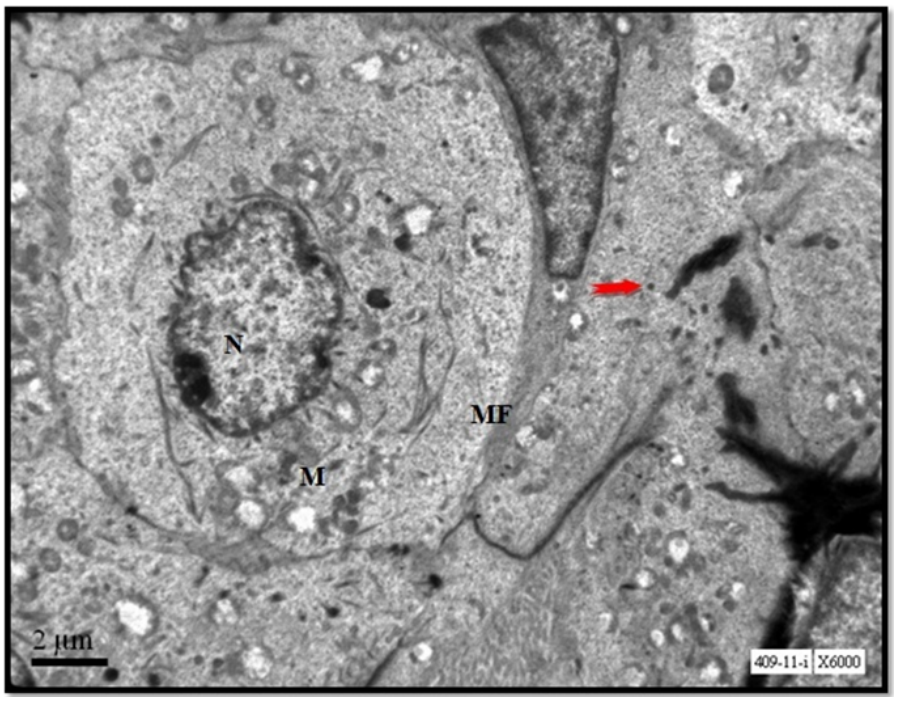

Fig 8. EM of rat ovary treated with fluoxetine 20mg/kg with inconspicuous nucleus (N).

4 Discussion

The amount of serum estradiol and progesterone in group II is less compared to the control, suggesting a decline in the female hormones during the depression. Earlier researches support this observation and a decrease in estrogen levels is found to have an impact on mood and mental status by its effects on estrogen receptor (ER) β [27,28]. On treatment with fluoxetine at a dose of 10mg/kg, there has been an increase in hormone levels, suggestive of a recovery. However, when the treatment dose is increased to 20mg/kg, there is a decline in the hormone level. This indicates the dysfunctioning and the impairment of the ovaries at a fluoxetine dose of 20mg/kg. Studies reveal that chronic fluoxetine treatments suppress the circulating estrogen [29]. Research work on female goldfish (*Carassius auratus*) also reported that fluoxetine significantly reduced the expression of (ER) β1 mRNA [30]. Fluoxetine's effects to disrupt the female sexual responses and may involve its effects on progesterone in the midbrain VTA (ventral tegmental area) [31]. Chronic fluoxetine treatment (5mg/kg/day) completely blocked the increased secretion of corticosterone and progesterone in response to the acute fluoxetine challenge [32].

The variations in the ovarian hormones may be attributed to the structural degeneration of the ovaries as is observed by EM studies. The electron micrographs of rat ovaries in the control group I depicted the normal appearance of stromal cells. A distinct graafian follicle and ovarian follicle under developmental stages were seen in the ovum. The presence of an active Golgi complex, the nucleus with the eccentric nucleolus, intact mitochondria with distinct cristae and a dense matrix indicates a normal condition of the ovary. Animals in depressed group II show a distinct nucleus with the nucleolus in the follicle. However, there are hydrated and ballooned mitochondria with loss of cristae and also the number of mitochondria is more indicating higher synthesis of ATP. Thus, it can be derived that there are more energy requirements in states of depression. There is also the heavy accumulation of polyribosomes indicating heavy protein synthesis. Animals undergoing treatment in group-III with 10mg/kg fluoxetine shows a distinct nucleus. Primary ovarian follicle undergoing degeneration and several hydrated mitochondria with loss of cristae are also observed. The presence of autophagic vesicle and membrane blebbing is indicative of some degenerative changes in the structure.

Female rats undergoing treatment with a dose of fluoxetine at 20mg/kg in group-IV show an inconspicuous nucleus and the appearance of several hydrated mitochondria which indicates damage to the organelle. There are reports of fluoxetine inducing a change in the mitochondrial membrane permeability and formation of reactive oxygen species, leading to cell death or apoptosis [33]. There is an invasion of the sterocilia into the stroma of the follicle which destroys the characteristic structure of the ovarian follicle. An increase in the number of atretic follicles, oocyte fragmentation, damaged zona pellucidas have been

reported in animals treated with fluoxetine [34,35]. A dose of flouxetine drug has also been found to reduce the number of graafian and prenatal follicles and corpus luteum, which reflect trauma in ovarian tissue [36].

5 Conclusion

Fluoxetine treatment at a dose of 20mg/kg results in degenerative changes in the ovaries of female rats by inducing a fibroid condition as is evident from the electron micrographs. The significant decrease in the levels of estradiol and progesterone reveals the dysfunctioning of the ovaries at 20mg/kg and not at 10mg/kg fluoxetine treatment.

The study concludes that fluoxetine having a wider therapeutic index for major depressive disorders causes impairment to the ovarian tissues in female rats. Fluoxetine therapy given to human females may as well exhibit a similar effect on the ovaries. Further research is essential to understand the effects of fluoxetine on gametogenesis in the female ovary.

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