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ORIGINAL ARTICLE

A Preliminary Study of Sex Differences and Histopathological Observations in Experimentally-Induced Alzheimer's Disease Albino Rats with Letrozole Administration.

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ABSTRACT

Background: Letrozole is an aromatase inhibitor with conflicting studies reporting its effects on memory in male and female animals, and whether the observed memory impairments become exacerbated with an additional insult such as Alzheimer's disease (AD); an illness in which beta-amyloid and hyperphosphorylated Tau (p-Tau) protein accumulate. We examined Letrozole effects on memory, hippocampal p-Tau and histopathology in animals treated with Letrozole only or on top of induced AD, and investigated whether there would be any sex-based differences in memory status and/or p-Tau.

Methods: Fifty-one adult male and female Sprague Dawley rats were divided into: Control groups: Intact, Letrozole Vehicle, CSF, and CSF+Letrozole Vehicle, Letrozole group, Streptozotocin (STZ) group; with induced AD via cerebroventricular STZ injection in artificial CSF and, STZ+Letrozole (STZ-L) group. Memory was tested in a T-maze to measure alternation percentage. Hippocampal p-Tau protein levels were estimated using ELISA, and histopathology was documented in sections stained with Hematoxylin and Eosin.

Results: There were significant reductions in alternation percentage in the STZ, Letrozole and STZ-L groups; most severe in the STZ-L group and least severe in the Letrozole group. Significant elevation in p-Tau concentration was observed in the STZ, Letrozole and STZ-L groups compared to the controls. No differences were found between males and females in all groups in any of the studied parameters. Histopathological examination showed neurodegeneration and inflammation in the Letrozole, STZ and STZ-L groups.

Conclusions: Letrozole alters memory, more so in experimentally-induced AD, increases p-Tau and may cause hippocampal pathological changes, with no sex-based differences.

Key words: Letrozole; Alzheimer's disease; Tau

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative illness with extracellular amyloid plaques and intracellular neurofibrillary tangles of hyperphosphorylated Tau as hallmarks required for definitive diagnosis. Activated microglial cells and reactive astrocytes can also be detected (1). Various studies found a higher prevalence of AD in females, with a more aggressive picture (2, 3). The higher prevalence of AD in women may be attributed to women living longer, on average, and Yue et al. suggested that estrogen deficiencies in the brains of AD women may be a main risk factor. However, the role of sex in AD is unclear and controversial. Studies of sex differences in regards to AD incidence in the USA have observed no differences in the rates of AD development between males and females (3). Controversies also exist in experimental studies, with reported sex variations in rodent models of

learning and memory (5), and in transgenic AD mice (6).

Letrozole is a highly selective, potent aromatase inhibitor that inhibits estrogen production and can cross the blood brain barrier (7).Since estrogen promotes the development and maintenance of different cognitive abilities (8); drugs that suppress aromatase may harmfully affect cognition (9).However, the effects of aromatase inhibitors (AIs), including Letrozole, on memory are controversial. Some studies with intact male rats suggest that AI treatment might improve working and spatial memory (10, 11). In contrast, Nayebi et al. showed that administration of Letrozole significantly impaired memory in intact rats.

Mixed results have also been reported in females, studies on females' mice and hippocampal cultures have shown that Letrozole suppressed learning and memory (13, 14). However, in the study by Aydin et al. it seemed that inhibition of brain estrogen synthesis may have positive effects on spatial memory.Human studies also have contradictory results. Cognitive side effects, such as concentration difficulties and forgetfulness, are reported among women treated with AIs (16), and meta-analyses of breast cancer patients treated with AIs revealed verbal learning and memory impairments in such patients (17). Conversely, others found no cognitive affection in women receiving AIs (18).It is also still uncertain whether Letrozole exacerbates the cognitive impairments in presence of an additional aging-associated insult, such as an increase of pathologic beta-amyloid (19). In addition, the possible effects of Letrozole on hyperphosphorylated Tau levels and hippocampal histology are underexplored.

This study aims to examine the effects of Letrozole on memory, hippocampal hyperphosphorylated Tau and histopathological picture in male and female rats treated with either systemic Letrozole alone or Letrozole on top of an existing experimentally-induced Alzhimer's disease, and to investigate whether or not there would be any sex differences in regards to memory status and/or levels of phosphorylated Tau.

Methods: Animals and grouping: Fifty-one adult male and female Sprague Dawley rats, similar in age and weight (150-200 gm.) were obtained from the medical experimental research center, Mansoura University. This study was ethically approved by the Institutional Animal Care and Use Committee, Zagazig University (Approval number: ZU-IACUC/3/F/83/2018), and all animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals. This study was carried out from June 2019 to November 2019.

Males and females were separately kept in plastic rat cages (3-5/cage) under controlled temperature (25±2°C), and a 12-hour light/ 12-hour dark cycle and had free access to food (standard commercial rat chow) and water. After 2 weeks of acclimatization, rats were randomly divided into the following seven groups: 1) *Intact Control* (n=6, 3 males, 3 females), 2) *Letrozole* (n=9, 5 males, 4 females): A daily dose of 2.5 mg/kg (10) of Letrozole (Synthon, Netherlands) dissolved in 0.5% carboxy methylcellulose (CMC) solution was orally administrated (15) for two weeks (13), 3) *Letrozole Vehicle (LV) Control* (n=6, 3 males, 3 females): Animals were orally given 0.5% CMC solution daily, for two weeks, 4) *STZ* (n=9, 5 males, 4 females): An Alzheimer's disease-like state was experimentally induced via intracerebroventricular injection (ICV) of a single 3 mg/kg dose of Streptozotocin (Sigma-Aldrich, USA) (20) in 10 µL artificial cerebrospinal fluid (aCSF) prepared following the recipe of Cold spring harbor, (2011), 5) *CSF Control* (n=6, 3 males, 3 females): aCSF was administrated by ICV injections., 6) *STZ + Letrozole (STZ-L)* (n=9, 5 males, 4 females): An Alzheimer's disease-like state was induced as before mentioned. Rats were allowed 7 days to recover then given 2.5 mg/kg of oral Letrozole for two weeks, and, 7) *CSF + Letrozole Vehicle (CSF-LV) Control* (n=6, 3 males, 3 females): aCSF was administrated by ICV injections. Rats were allowed 7 days to recover then orally given 0.5% CMC solution daily, for two weeks.

Induction of AD by ICV injection of STZ

Animals were anesthetized with Ketamine (75 mg/kg, intraperitoneal) and Xylazine (5 mg/kg, intraperitoneal), then each positioned in the stereotaxic frame and two bilateral holes were made through the skull using coordinates according to the rat stereotaxic brain atlas (22): Anteroposterior from bregma= - 0.9 mm, Mediolateral from the midline= ± 1.5 mm and Dorsoventral from the skull= 3.6 mm. Streptozotocin dose was injected bilaterally into the lateral ventricles using a Hamilton syringe at a rate of 0.2 µL/min (12). Rats were allowed 7 days to recover before any additional treatment. STZ effects on memory typically begin to appear two weeks after injection (20).

Testing of working memory: Spontaneous alternation in a modified T-maze

A manually-run maze was built as designed by Deacon and Rawlins, (2006). Habituation to the maze was not allowed prior to testing. Per animal, a sample run and five choice trials were done each day for two consecutive days, i.e. a total of 12 trials/rat, and 10 possible alternations. In the sample run, each rat was placed in the starting area

at the bottom of the T and allowed to select a goal arm. The rat was kept for 30 seconds in the selected arm, and then it was removed and put back in its cage for an intertrial interval of 10 minutes. In the choice runs, the central partition was removed, and the rat was placed again at the starting point, and allowed to pick one of the two open goal arms. Alternation was defined as: When the rat chose the opposite arm of that chosen in the previous run. A percentage correct choice (alternation) per rat was calculated as follows: (No. of correct choices/ Total possible alternations) x 100 (23, 24).

Biochemical testing and histopathological examination

Rats were sacrificed by cervical dislocation according to the University of Texas at Austin (2013) guideline. The brain was removed and hippocampi were dissected as previously described (26). Each hippocampus was immersed in sterilized phosphate buffered saline (PBS) and kept at -80°C (27).

Rat hyperphosphorylated Tau (Ser396) protein concentration in the hippocampus was measured using an ELISA kit (Bioassay technology laboratory, Shanghai, China) according to the producer's instructions.

For histopathological examination, representative hippocampi were fixed in formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and then embedded in paraffin wax. Paraffin sections of 5 µm thickness were stained with Hematoxylin and Eosin (H&E) (28).

Statistical analysis

IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA) was used. Mean and standard deviation were calculated for quantitative variables. One way analysis of variance (ANOVA) test was used to compare means of multiple groups in parametric data. The difference between quantitative variables in two groups in parametric data was calculated using T-test. For all the stated tests, P values <0.05 indicated statistical significance, while P values >0.05 indicated non-significance.

RESULTS

Comparing sex distribution in the studied groups

Table (1) shows the comparison of sex distribution among the different studied groups. There were no statistically significant differences between the different groups in sex distribution.

Comparison between male and female rats in the different studied parameters among the studied groups

Table (2) shows that there were no statistically significant differences between male and female rats in all groups in any of the studied parametersComparing memory status (in alternation percentage) and p-Tau levels (in pg/ml) among the different studied groups

Regarding the alternation percentage, **figure (1)** shows that there were statistically significant decreases in alternation percentage in the STZ, Letrozole and STZ-L groups compared to the control groups (i.e., Intact, CSF, LV and CSF-LV). Between the Letrozole, STZ and STZ-L groups, the decrease in the alternation percentage was most severe in the STZ-L group (17.78 ± 4.41) and least severe in the Letrozole group (53.33 ± 8.66), while the STZ group had a mean ± SD of 35.56 ± 8.82. Compared to each other, the differences in alternation percentage between each two of the STZ, Letrozole and STZ+L groups were statistically significant.

Concerning p-Tau, **figure (2)** shows that there was a statistically significant elevation inconcentration in the STZ (mean ± SD: 22.36 ± 0.83), Letrozole (23.24 ± 1.23) and STZ-L (23.37 ± 1.46) groups each compared to the control groups. However, no difference was found between STZ, Letrozole and STZ-L groups in regards to p-Tau concentration when compared to each other.

Figure (1): Alternation percentage in the studied groups

Table (1): Sex distribution in the studied group.

Parameter / *Groups*	Male N (%)	Female N (%)
Intact (n=6)	3 (50%)	3 (50%)
CSF (n=6)	3 (50%)	3 (50%)
LV (n=6)	3 (50%)	3 (50%)
CSF-LV (n=6)	3 (50%)	3 (50%)
STZ (n=9)	5 (55.6%)	4 (44.4%)
Letrozole (n=9)	5 (55.6%)	4 (44.4%)
STZ-L (n=9)	5 (55.6%)	4 (44.4%)
χ^2	**0.16**	
P	**0.99 NS**	

Symbols and abbreviations: χ^2: Chi square test, NS: Non significant (P>0.05)

CSF: Cerebrospinal fluid, STZ: Streptozotocin, L: Letrozole, LV: Letrozole vehicle.

Table (2): Comparison between males and females in all studied parameters.

Group	Variable		N	Mean	SD	Median	Range		Test	P
Intact	**Alternation percentage**	**Male**	3	73.33	15.28	73	60.00	90.00	t	
		female	3	80.00	10.00	80	70.00	90.00	0.63	0.56 NS
	p-Tau concentration (pg/ml)	**Male**	3	17.37	3.26	17.27	13.61	19.32	t	
		female	3	16.34	2.80	16.40	13.60	19.19	0.39	0.71 NS
CSF	**Alternation percentage**	**Male**	3	76.67	15.28	77	60.00	90.00	t	
		female	3	86.67	15.28	87	70.00	100.00	0.80	0.47 NS
	p-Tau concentration (pg/ml)	**Male**	3	17.98	2.77	18	14.78	19.71	t	
		female	3	18.59	3.35	18.5	14.80	21.14	0.24	0.82 NS
LV	**Alternation percentage**	**Male**	3	70.00	10.00	70	60.00	80.00	t	
		female	3	76.67	5.77	77	70.00	80.00	1.00	0.37 NS
	p-Tau concentration (pg/ml)	**Male**	3	16.85	2.70	17	13.74	18.54	t	
		female	3	18.55	1.01	18.5	17.38	19.20	1.02	0.36 NS
CSF-LV	**Alternation percentage**	**Male**	3	76.67	15.28	77	60.00	90.00	t	
		female	3	80.00	10.00	80	70.00	90.00	0.32	0.77 NS
	p-Tau concentration (pg/ml)	**Male**	3	18.75	0.87	18.5	18.00	19.70	t	
		female	3	19.03	0.89	19	18.50	20.05	0.39	0.72 NS
STZ	**Alternation percentage**	**Male**	5	34.00	8.94	34	20.00	40.00	t	
		female	4	37.50	9.57	37.5	30.00	50.00	0.57	0.59 NS
	p-Tau concentration (pg/ml)	**Male**	5	22.52	0.94	22.5	21.79	23.61	t	
		female	4	22.16	0.75	22	21.14	22.73	0.63	0.55 NS
Letrozole	**Alternation percentage**	**Male**	5	50.00	10.00	50	40.00	60.00	t	
		female	4	57.50	5.00	57.5	50.00	60.00	1.36	0.22 NS
	p-Tau concentration (pg/ml)	**Male**	5	22.74	1.01	22.5	21.10	23.87	t	
		female	4	23.86	1.31	24	21.90	24.53	1.46	0.19 NS
STZ-L	**Alternation percentage**	**Male**	5	7.65	2.73	8	10.00	20.00	t	
		female	4	18.00	4.47	18	10.00	20.00	0.16	0.88 NS
	p-Tau concentration (pg/ml)	**Male**	5	17.50	5.00	18	21.66	24.40	t	
		female	4	22.81	1.45	23	22.70	25.20	1.36	0.22 NS

Symbols and abbreviations: t: Independent t test, NS: Non significant (P>0.05)

CSF: Cerebrospinal fluid, STZ: Streptozotocin, L: Letrozole, LV: Letrozole vehicle, p-Tau: Phosphorylated Tau

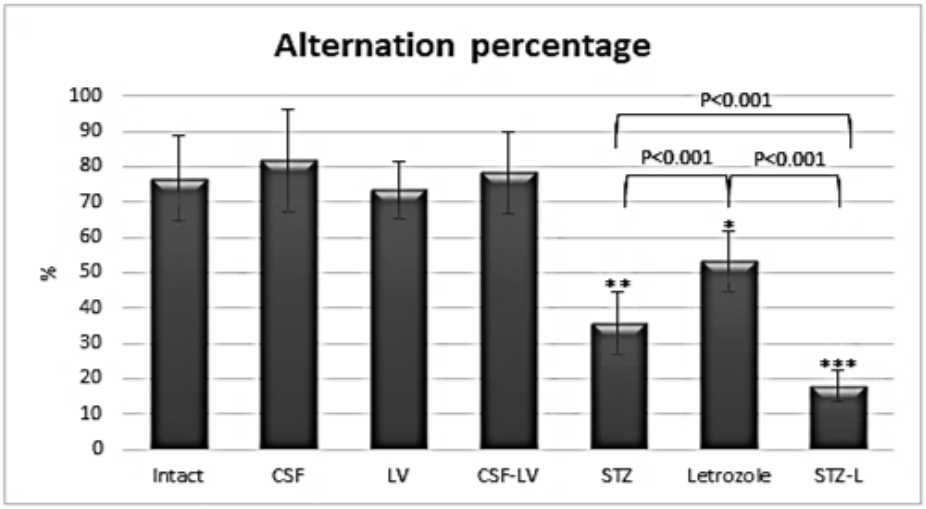


Figure (1): Alternation percentage in the studied groups.
***, ** & ***:** Significantly different than controls (P value<0.05), CSF: Cerebrospinal fluid, STZ: Streptozotocin, L: Letrozole, LV: Letrozole vehicle.
STZ, Letrozole & STZ+L groups showed significant reductions in alternation percentage compared to the controls. Among the STZ, Letrozole & STZ+L groups, the severity of decrease in alternation percentage significantly varied, with STZ+L group showing the most severe decrease and Letrozole group showing the least.

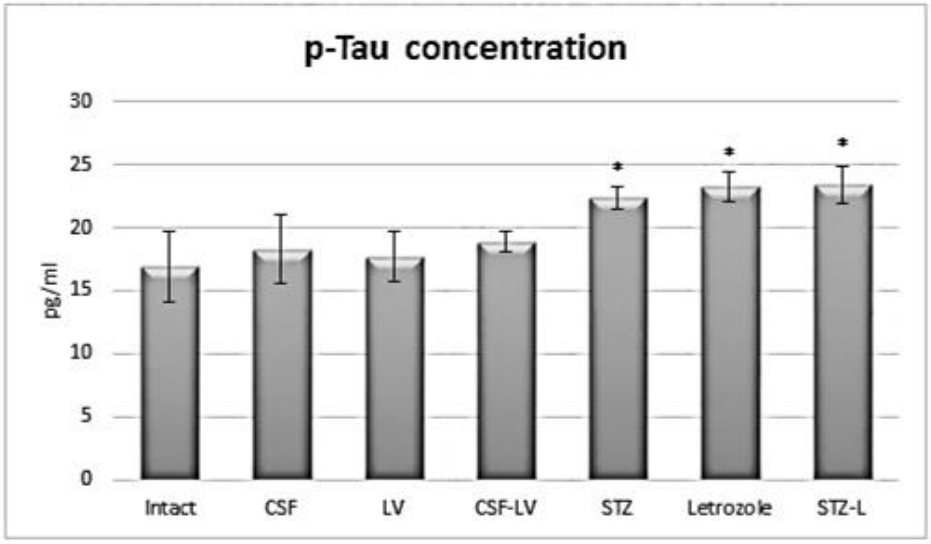
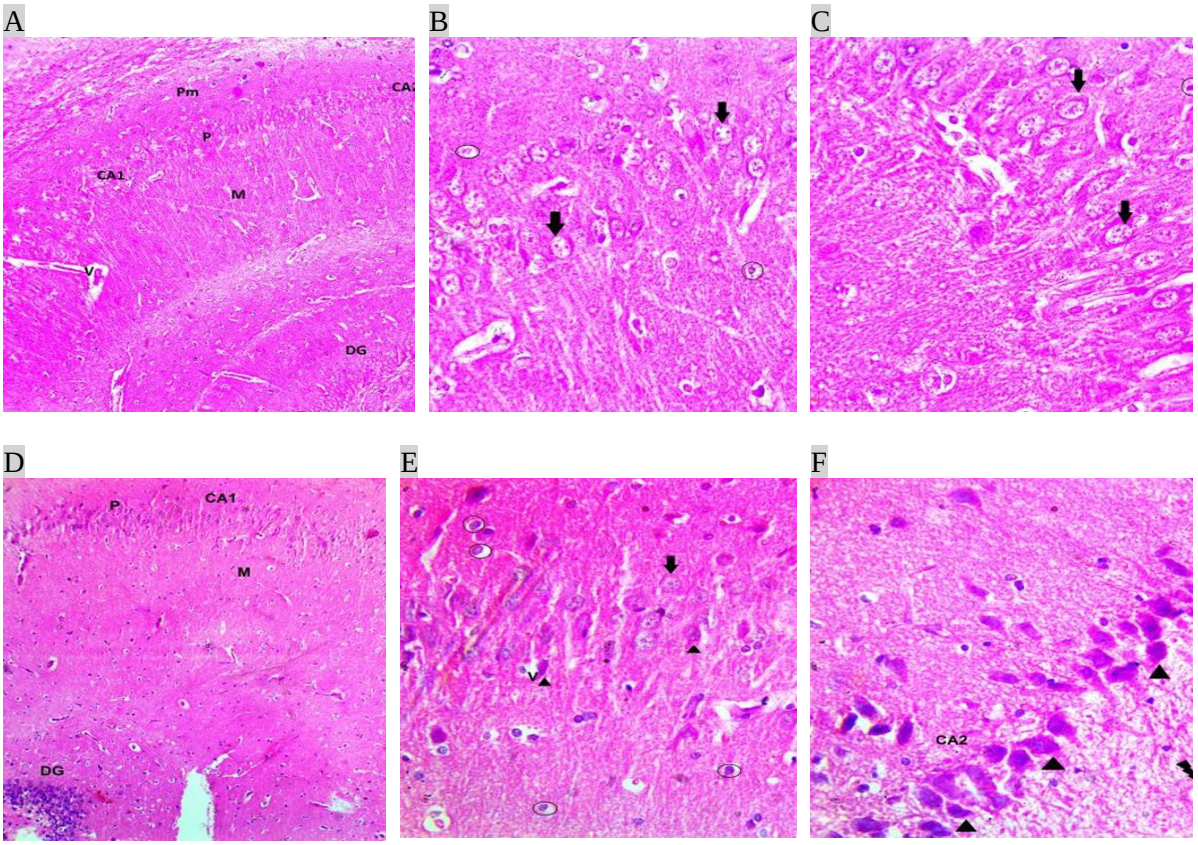


Figure (2): p-Tau concentrations in the studied groups .
***** Significantly different than controls (P value<0.05). CSF: Cerebrospinal fluid, STZ: Streptozotocin, L: Letrozole, LV: Letrozole vehicle, p-Tau: Phosphorylated Tau.
STZ, Letrozole and STZ+L groups showed significant elevations in the levels of p-Tau compared to the controls. No significant difference was found between the STZ, Letrozole and STZ+L compared to each other.



Histopathological examination of random samples

Figure (3) shows degeneration of pyramidal cells with evident neurofibrillary tangles (NFTs), cellular loss, increase and enlargement of astrocytes and amyloid depositions in the STZ and STZ+L groups compared to the controls.

In the specimens taken from the Letrozole-only group, some degenerated pyramidal cells, with and without NFTs, excess and enlarged astrocytes and minimal deposition of amyloid were shown.

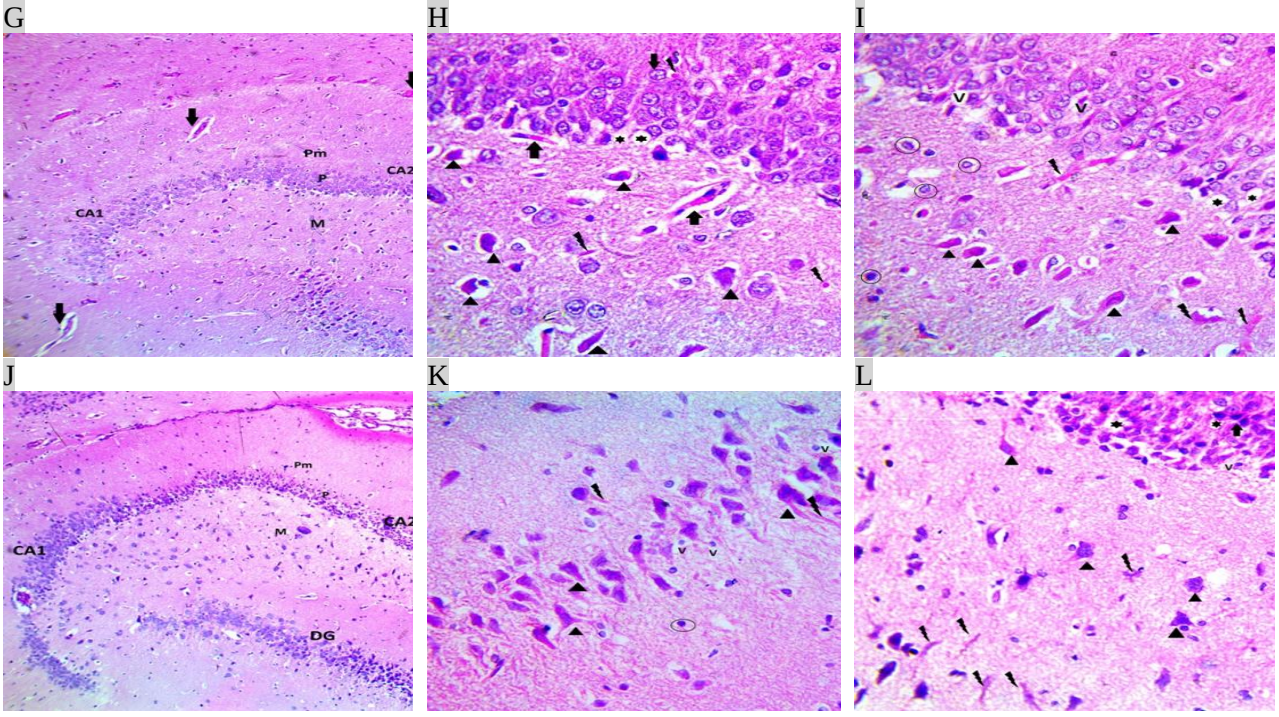


Figure (3): Histopathological examination of hippocampal sections stained with Hematoxylin and Eosin (H&E).

A-C Show sections from control groups: A) Shows Cornu ammonis (CA), Dentate gyrus (DG), Molecular layer (M), Pyramidal layer (P), Polymorphic layer (Pm), and blood vessels (V) (H&E x100). **B)** CA1 showing normal pyramidal cells (arrows) with normal thickness of the pyramidal layer, Astrocytes (circles) are found in the molecular layer and in the polymorphic layer (H&E x400). **C)** CA3 showing normal thickness of the pyramidal layer with normal pyramidal cells (arrows), Astrocytes (circles) are found in the molecular layer and in the polymorphic layer (H&E x400).

D-F Show sections from the Letrozole (L) group: D) Shows Cornu ammonis (CA), Dentate gyrus (DG), Subiculum, (S), Molecular layer (M), Pyramidal layer (P), Polymorphic layer (Pm) (H&E x100). **E)** CA1 with degenerated pyramidal cells (arrow heads) surrounded by pericellular haloes within the normal pyramidal cells (arrows) and some vacuolated cells (V). Enlarged and excess astrocytes (circles) are seen in the molecular (downward) and the polymorphic (upward) layers & minimal amyloid deposition (broken arrows) in the molecular layer (H&E x400). **F)** CA2 showing degenerated pyramidal cells with neurofibrillary tangles (arrow heads) with decreased thickness of the pyramidal layer. Minimal deposition of amyloid protein (broken arrows) is seen in the molecular layer (H&E x400).

G-I Show sections from the STZ group: G) Shows increased cellularity in the molecular layer and the polymorphic layer and increased vascularity (wide and thick walled blood vessels, arrows) (H&E x100). **H)** CA2 showing degenerated pyramidal cells with NFTs (arrow heads) and cell loss (asterisks) within the normal pyramidal cells (downward arrows). Many degenerated pyramidal cells with NFTs (arrow heads) in the molecular layer, as well as deposition of amyloid protein (broken arrows) and widened and thickened blood vessels (upward arrows) (H&E x400). **I)** CA2 showing many degenerated pyramidal cells with NFTs (arrowheads), cell loss (asterisks) and vacuolated cells (V). Many degenerated pyramidal cells with neurofibrillary tangles (arrowheads) are seen in the molecular layer as well as excess astrocytes (circles) and deposition of amyloid protein (broken arrows) (H&E x400).

J-L Show sections from the STZ-L group: J) Shows marked increase of cellularity (H&E x100). **K)** CA2 showing many degenerated pyramidal cells with neurofibrillary tangles (arrow heads) and vacuolated cells (V). Enlarged and excess astrocytes (circles) are found in the molecular layer (downward) and the polymorphic layer (upward) with deposition of amyloid protein (broken arrows) (H&E x400). **L)** CA2 showing many degenerated pyramidal cells (arrows) in the pyramidal layer, cell loss (asterisks) and vacuolated cells (V). All pyramidal cells in the molecular layer are degenerated with neurofibrillary tangles (arrow heads). Deposition of amyloid protein is also seen (broken arrows) (H&E x4)

DISCUSSION

Alzheimer's disease is a neurodegenerative illness with beta-amyloid plaques and neurofibrillary tangles of filamentous Tau proteins (1). Incidences of AD were reported to be higher in females, with a more aggressive picture (2,3). However, the role of sex in AD is still controversial.

Letrozole is an aromatase inhibitor capable of crossing the blood brain barrier (7), and there is controversial data regarding the effects aromatase inhibitors on memory in both male and female rodents (10,12,13,15).In this study we examined the effects of Letrozole on memory, hippocampal hyperphosphorylated Tau and histopathological picture in male and female animals treated with either systemic Letrozole alone or Letrozole on top of an existing experimentally-induced Alzhimer's disease.Our results regarding memory impairments in the STZ, Letrozole and STZ+L groups are somewhat in agreement with Nayebi et al., who also stated the presence of memory impairment in the STZ, Letrozole, STZ+Letrozole groups, with the Letrozole group being the least affected. However, they found no difference between the STZ+Letrozole group compared to the STZ only group. Differences could be due to variations in the animals used; as they used the Wistar strain, the dosage and route of administration of Letrozole; as they opted for a 6 mg/kg dose/intraperitoneally, or, the usage of another memory test, i.e. passive avoidance.

In regards to the p-Tau (Ser396) level, our results showed that it significantly increased in the STZ, Letrozole and STZ+Letrozole groups, with no differences between these three groups and with no differences between male and female animals in any group.

Hyperphosphorylated Tau generation after ICV-STZ administration was widely documented in previous studies (29–33). However, the potential effects of Letrozole on p-Tau levels are not extensively studied. The results showed a significant elevation of p-Tau in the Letrozole group compared to the control groups, which is not an entirely unexpected finding since previous studies have shown that estrogen prevents Tau hyper-phosphorylation in neuroblastoma cell lines and primary cultures of rodent cortical neurons (34), and that ovarictomy increased the age-associated Tau hyperphosphorylation in the hippocampus (35).Interestingly, the current results showed no significant differences in the levels of

p-Tau between the STZ, Letrozole and STZ+Letrozole group compared to each other. The levels of the measured p-Tau did not exactly mirror the degree of memory impairment in the STZ, Letrozole and STZ+L groups. This could be explained by the fact that we only measured phosphorylated Tau at residue Ser396, while there are more than 70 potential Tau phosphorylation sites spanning almost the whole structure of the protein, many of which are of pathological relevance (29,30,32,36).

Histopathological examination results showed signs of neurodegeneration, amyloid depositions and inflammation in the specimens taken from the STZ and STZ+L groups compared to the controls. These results are in agreement with multiple previous studies (37–40). In the specimens taken from the Letrozole-only group, histopathological examination revealed the presence of some degenerated pyramidal cells, with and without NFTs, excess and enlarged astrocytes and minimal deposition of amyloid. Studies showing histological effects of Letrozole on the hippocampus are scarce, and our observations seemingly contradict those of Iqbal et al., who found no effect of Letrozole on the normal histology of the hippocampus. However, this current study did not examine whether or not the histopathological findings were of statistical significance. Cognitive and hippocampal p-Tau results of the current study showed no significant differences between male and female rats in any of the studied groups, which contradicts findings from animal studies that showed gender differences in memory and learning tests (5). However, it can be argued that differences in animal models, tests and timing of them could lead to differential results. Even in the same model, the time at which the animal is tested for cognitive impairment after STZ injection might yield different results as there is an acute decline in cognitive functions observed 2 weeks to 1 month of STZ injection, followed by partial compensation until 3 months, then the compensation fails and a slow and progressive memory decline takes place (42).

Our results showed that both males and females demonstrated memory impairments in response to STZ, Letrozole and STZ+Letrozole administration compared to the control groups with no significant differences between males and females in regards to the resulting memory impairment.

For the STZ group, only a single recent study examined the possible differences between males and females in the induction of an AD-like state (43). In their study, Bao et al. reported that ICV-STZ resulted in learning and memory impairment in the Sprague Dawley male rats, but not in female rats, contrary to the results of our study which showed that both sexes demonstrated comparable degrees of memory impairment following STZ injection. This discrepancy could be explained by the use of different tests to assess the degree of memory impairment, as Bao et al. used the Morris water maze (MWM), in contrast to the T-maze used in the current study. Some authors suggest that the T-maze might be superior to the MWM in detecting hippocampal dysfunction (23).

In the Letrozole-only group, the results showed that both male and female rats exhibited significant memory impairment in comparison to the control groups, and the degree of impairment was comparable between males and females with no significant differences. The finding that Letrozole impaired the memory in females is in agreement with various studies (13,44–47). However, Aydin et al. presented different findings, as inhibition of aromatase enzyme over 6 weeks did not significantly change spatial learning performance of intact rats, and improved memory consolidation in rats administrated Letrozole in an oral dose of 1 mg/kg.

The study design of Aydin et al. was different from ours, as our Letrozole dose was higher (2.5 mg/kg, oral gavage) and given for a shorter period (2 weeks), they also used MWM for memory testing in contrast to the spontaneous alternation in a T-maze which was completed in a much shorter period (An hour per day for two days independent of each other, compared to the MWM procedure that entails a 5-days acquisition phase and a 6th day probe trial). Previous studies indicate that rapid learning and retention for spatial information over a relatively short interval is not affected by the normal cyclic hormonal changes in the female rats (48).In Letrozole treated male rats, the results were in agreement with Nayebi et al., whose results also showed memory impairment in male rats with Letrozole administration. On the other hand, the results contradicted those of Moradpour et al., who showed a significant improvement in memory of the treated rats compared with the controls, but, only at a certain dose of the drug. However, in comparison to the current study, their work was conducted using a different rat strain (Wistar), memory test (MWM) and aromatase inhibitor (Anastrazole) with a different administration route (Direct intrahippocampal). Although Anastrozole and Letrozole are both triazoles, studies suggests that they are not equipotent, and preclinical and clinical evidence indicates different pharmacological profiles (49).

The current results also contradicted those of (10), who used adult male Sprague Dawley rats to test for spatial working memory in a cross arm maze,

following Letrozole administration. They found that Letrozole improved the acquisition of working memory in their animals.

Differences in findings between the two studies might be explained by the use of a different memory task, and animals with different ages. Alejandre-Gomez et al. used animals weighing 250-300 gm. at the time of testing, which are relatively older than the animals used for this study; which weighed approximately 200 gm. at the time of the experiments. Testosterone, which increases with the use of aromatase inhibitors improves spatial working memory in aged male rats (50).

CONCLUSIONS

Letrozole alone was sufficient to show a significant degree of memory impairment, more pronounced in the presence of an existing insult, and alter the level of p-Tau in the hippocampus, with possible effects on its histopathological picture.

Additional research is needed to establish an expected histopathological picture to look for when examining the hippocampi of Letrozole-only treated animals.

We also recommend the inclusion of larger samples sizes, a battery of different behavioral tests repeated on timed intervals over a long period, and multiple biochemical and histopathological parameters in the future studies examining the sex differences in response to STZ administration or Letrozole treatment, as we consider the small sample size used in the males versus females comparisons to be a limitation of our study.

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