

ORIGINAL ARTICLE

Histomorphological findings in the brain of adult Wistar rats exposed to the ecstasy Pill; 3, 4 methylenedioxymethamphetamine

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ABSTRACT

In all vertebrates and the majority of invertebrates, the brain is the organ that acts as the hub of the nervous system. 3,4-methylenedioxymethamphetamine (MDMA), a derivative of amphetamine that affects the central nervous system (CNS), is marketed under the brand name Molly and is abused for its intoxicating recreational effects. Using adult Wistar rats, this study sought to ascertain the histomorphological effects of molly on the brain. The animal farm provided 25 adult Wistar rats with a mean weight of 110.10g, and segmented into five groups (A–E). Groups B through E were the test groups, and group A was the control. Molly was made in graded doses for Groups B through E, and the amount to be given was determined by weighing the dosages. Sections were taken from the samples and subjected to histological processing after brain tissues were removed and preserved in 10% formal saline for a full day. A segment of the brain including normal neurons with normochromic nuclei, pale cytoplasm, and normal glial cells was displayed in groups A (control), B, and E. There were typical fibrillary extensions in the backdrop. In conclusion, modest dosages of MDMA did not cause alterations in the brain associated with oxidative stress. Adult rats treated with MDMA did not exhibit changes in brain morphology or histology. To determine whether moderate MDMA dosages cause brain alterations in adult animals and whether these changes are permanent and might manifest later in maturity or old age, more research is needed.

Keywords: Brain, Drug, Methylenedioxymethamphetamine, Molly, Wistar Rat

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INTRODUCTION

The brain is the most intricate organ in vertebrates, with the human cerebral cortex housing 15–33 billion neurons. Each neuron forms thousands of connections with others through synapses, enabling communication via axons that transmit action potentials to specific target cells [1].

MDMA, commonly known as molly or ecstasy, is an amphetamine-based substance that impacts the central nervous system (CNS) and is frequently abused for its psychoactive effects [2]. Available in pill or powder form, MDMA abuse has been linked to numerous adverse effects on the body, including inflammation, necrosis, oxidative damage to cellular components such as lipids, proteins, and DNA, as well as disruptions in antioxidant enzyme activity [3]. Acute misuse of the drug can result in severe complications like cardiac arrhythmias, hypertension, hyperthermia, liver damage, serotonin syndrome, coma, and even death in extreme cases [2].

Globally, approximately 1.2% of the population (24.7 million people) is estimated to use MDMA, with high prevalence rates in regions like the Middle East, Asia, Oceania, and North America. The drug is particularly popular among young adults aged 18–35, with 6–12% having reported using it at least once in their lifetime. Despite its widespread use and increasing prevalence, especially among young, fertile individuals, an effective pharmacological treatment for MDMA abuse remains unavailable [2].

For decades, amphetamine-type stimulants, including 3,4-methylenedioxymethamphetamine (MDMA, commonly known as "ecstasy"), have been widely used recreationally by adolescents and young adults worldwide [3–4]. In 2012, the United Nations Office on Drugs and Crime estimated that 9.4 to 28.2 million individuals globally consumed MDMA [5]. Regional data showed that in 2010, 2.0 out of 2.5 million "ecstasy" users in Europe were between 15 and 34 years old, while in the United States, 2.5 out of 2.6 million users the previous year were aged 14 to 34 [5]. Recreational use of MDMA often involves binge consumption, characterized by multiple doses taken within a short period [6–8].

Research with adolescent animal models has revealed that neurotoxic doses of MDMA can result in long-term deficits in memory, learning, and the serotonergic system

[3, 9–10]. These findings underscore the importance of adolescent-focused studies, as ethical constraints make it challenging to study drug toxicity in human adolescents. Nonetheless, studies on young human populations indicate cognitive deficits [14], reduced grey matter concentration [13], and serotonergic system impairments following MDMA misuse [11–12, 15].

MDMA neurotoxicity is known to be dose-dependent, with researchers requiring specific doses to induce neurotoxicity in laboratory animals [3–4]. In rats, a single neurotoxic dose typically ranges from 20 to 40 mg/kg, but cumulative doses can be much higher when administered over several days [4]. While these high doses are essential for investigating neurotoxicity, they often exceed the typical exposure levels of average MDMA users, aligning more closely with heavy abuse patterns. Using allometric scaling, researchers can estimate equivalent doses for humans: $\text{Human dose (mg/kg)} = \text{animal dose (mg/kg)} \times (\text{animal weight/human weight})^{1/4}$ [16]. Although this method provides an approximate equivalence, it does not account for differences in MDMA metabolism or administration routes between humans and rats.

According to this relationship, a daily dose of 40 mg/kg in adult rats equates to approximately 700 mg for a 70-kg human. Recent European Union reports indicate that MDMA tablets typically contain 57 to 102 mg [17], meaning consuming more than seven tablets in a single session would represent extreme use. In our study, we selected a binge-dosing regimen in adult Wistar rats (10 weeks old) consisting of three doses of 5 mg/kg MDMA, administered intraperitoneally (i.p.) every two hours. This dosing pattern has been shown to induce long-term serotonergic neurotoxicity [18]. However, in adolescent Wistar rats (postnatal day 40), no significant 5-HT depletion was observed in any of the four brain regions examined seven days after MDMA administration (manuscript in preparation). Based on the binge-dosing pattern, where typical "ecstasy" abusers consume two to four tablets per session [6, 8], our procedure's dose corresponds to the consumption of two to three tablets by adolescent users. This aligns with current data on typical binge-dosing behavior.

European Union report on drugs. As a result, the current paradigm for exposing teenage animals attempts to replicate the dosage regimen that adolescents in humans employ.

Studies with more moderate dosages that closely resemble those of the average MDMA user are therefore required. It is estimated that around 50% of cases of infertility are caused by male causes. Since earlier tests that examined Wistar rats for a shorter duration of seven days did not reveal any serotogenic damage, the research gap that this work aims to fill speaks to an increase in both dosage and duration of exposure to MDMA. Using adult Wistar rats as the animal paradigm, this study sought to ascertain the histomorphological effects of molly on the brain. Therefore, utilizing adult Wistar rats as the animal model, this study aims to ascertain the histomorphological effects of molly on the brain.

MATERIALS AND METHODS

Research Design

Wistar rats were employed as research subjects in this experimental study design. Twenty-five adult albino Wistar rats were used in the study; they were killed after receiving molly for 21 days. Following treatment, the rats were anesthetized with mild chloroform, and the heart was removed for histological examination. Brain samples were gathered and preserved for a full day in 10% formal saline. Sections of the brain were taken from the samples. In the cutting room, samples were sliced to a thickness of 3 mm. After being carefully tagged and subjected to histological processing, the chosen tissues were put in tissue baskets.

Ethical Consideration

Approval for the study was obtained from the Research Ethics Committee of the College of Medical Sciences (Ref No: AAU/HREC/23/1056) and was carried out in strict accordance with the guidelines for the care and use of animals for the research committee which is in line with that set by WHO [19].

Experimental Animals/Housing Condition

Following the standard guide for the care and use of laboratory animals, the Wistar rats that were purchased from the animal farm were moved to the experimental laboratory at the Department of Medical Laboratory Science's Histology laboratory, where they were given two (2) weeks to acclimate.

Experimental Animals and Grouping

To prevent contamination, the twenty-five (25) adult

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albino Wistar rats that were purchased, with a mean weight of 110.10g, were housed in wire mesh cages with tripods that kept the animal away from its excrement. The rats were fed growers' mash and given unlimited water during this acclimation phase.

The four-month-old adult Wistar rats used in the experiment were divided into five groups (A–E). There are five rats in each group ($n = 5$), and they are housed in five large cages. Groups B through E were the test groups, and group A was the control.

Molly was made in graded doses for Groups B through E, and the amount to be given was determined by weighing the dosages. Groups B, C, D, and E each received 0.12 mg/kg, 0.18 mg/kg, 0.32 mg/kg, and 0.40 mg/kg, respectively.

Group A was not given molly; instead, they were given water and the standard feed, which is farmer's mash.

Drug Preparation

The drug (molly) was bought from a government-approved pharmacy and diluted with commercially made normal saline to the proper concentrations. The animals were given the drugs orally using a 1.0 ml standard syringe for 21 days. Molly was ground, dissolved, and diluted with normal saline to approximate concentration. Each animal in groups B through E was selected one at a time using a hand towel, and the right amounts of the drugs were given to the animals orally.

Drug Administration

For this investigation, a total of twenty-five (25) adult Albino Wistar rats were employed. Five groups of five rats each were created from them. Before being given Molly and before being killed, the rats were weighed. Molly was administered orally in the manner described below:

For 21 days, Group A (Control) was given only regular feed (growers' mash) and purified water.

Growers' mash, graded amounts of molly (B, 0.12 mg/kg body weight each; C, 0.18 mg/kg body weight each; D, 0.32 mg/kg body weight each, and E, 0.4 mg/kg body weight each), and water were provided to groups B–E (test groups) at will for 21 days. There were two stages to the study: the acute phase and the chronic phase. Three (3) adult albino Wistar rats were slaughtered after 21 days of molly treatment, whereas two (2) were sacrificed after 10 days of molly administration for the

acute phase. Following dosing, the rats were anesthetized with mild chloroform, and their brains were removed for histological analysis.

Study Duration

The administration of MDMA to the test animals was conducted over a period of 21 days. However, the entire study, including preliminary investigations, animal acclimatization, procurement of materials (for MDMA preparation and production), the actual animal experiment, and the evaluation of results, spanned five months, from August to December 2015.

Sample Collection and Analysis

At the conclusion of each stage (10 days for acute phase and 21 days for chronic phase), the brain of each rat was taken under chloroform anesthesia and fixed in 10% formalin for histological processing. The animals' weights were measured before and after acclimatization, and comparable weight measurements were made at the end of each week, with the average weight being recorded accordingly.

Histological Processing

The tissues were treated using an automatic tissue processor in accordance with the Histology Laboratory's normal processing schedule. The tissues from the fixed plastic cassettes in 10% formalin were automatically processed by undergoing the following grades of alcohol processing:

70% alcohol	2hrs
80% alcohol	2hrs
90% alcohol	2hrs
90% alcohol	2hrs
95% alcohol	2hrs
Absolute	2hrs
Xylene 1	2hrs
Xylene II	2hrs
Molten paraffin wax 1	2hrs
Molten paraffin Wax II	2hrs

The tissues were taken out of their plastic cassettes after the final time, put in the middle of the metallic tissue mold, and then filled with melted paraffin wax. Additionally, they were allowed to firm before being refrigerated for 15 minutes at 5°C. A knife

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was used to remove the blocks from the metallic case once they had cooled in the refrigerator for the previously specified fifteen minutes. Next, the paraffin wax on the blocks' sides was removed.

A rotary microtome was then used to trim and cut serial pieces of the blocks at 3 nm. The parts were lifted using a clean frosted end slide after floating in a water bath at 55°C. Following a 40-minute exposure to a hot plate to ensure proper attachment of the sections, the frosted end slides were dewaxed, hydrated, allowed to air dry, and then placed in a slide box in preparation for the staining procedure.

Staining Procedure

The following is how the Haematoxylin and Eosin procedure was used to stain sections for general tissue structure:

1. The portions underwent two xylene changes (5 minutes) to remove the wax.
 2. Absolute, 95%, 80%, and 70% alcohol grades were used to hydrate the portions.
 3. Harris haematoxylin was used to stain the sections for five minutes.
 4. To get rid of extra stain, the parts were rinsed under running tap water.
 5. The portions were separated for three seconds in 1% acid alcohol.
 6. For ten minutes, the parts were blued in running tap water.
 7. 1% eosin was used to counterstain the portions for one minute.
 8. After being rinsed with water, the sections were dehydrated using increasing alcohol grades (70%, 80, 95%, and absolute).
 9. After being cleaned in xylene, the sections were allowed to air dry before being mounted using dibutylphthalate in xylene (DPX).
- Photomicrographs and an Olympus light microscope were used to analyze the slides.

Photomicrography

After examining the sections under a light microscope, each group's photomicrographs were obtained. After that, the photomicrographs are utilized to interpret each group's results.

Data Analysis

Following data collection, SPSS (version 25) was used for statistical analysis. ANOVA (Scheffe) was used to

compare the values of the test groups with those of the control group at a 95% confidence level.

RESULTS

(see table and figures in Appendix)

Results on Weight

The body weight variations of rats fed molly at different intervals are shown in Table 1. The findings demonstrated that throughout the trial, the control's weight grew. But in every group, the rats that were fed molly also gained weight. Rats in all groups had considerable body weight mean and standard deviation values before the molly administration phase.

Histomorphological Changes

Figure 1: Control-Group A: Normal neurons (thick arrow) with a normochromic nucleus and pale cytoplasm are visible in this section of the brain. Normal glial cells (thin arrow) are also present. There are typical fibrillary extensions in the backdrop. Higher magnification of the normal brain (H/E x400).

Figure 2: Group B: Normal neurons with a normochromic nucleus and pale cytoplasm are shown in this section of the brain (thick arrow). Normal glial cells (thin arrow) are also present. There are typical fibrillary extensions in the backdrop. Characteristics are consistent with typical brain tissue. Higher magnification of the normal brain (H/E x400).

Figure 3: The brain's Group C section displays typical neurons (thick arrow), which have a normochromic nucleus and pale cytoplasm. There are also typical glial cells (thin arrow). The usual fibrillary extensions are in the backdrop. An enlarged view of the normal brain (H/E x400).

Figure 4: Group D: Normal neurons with a normochromic nucleus and pale cytoplasm are visible in the brain section. Normal glial cells (thin arrow) are also present. There are typical fibrillary extensions in the backdrop. Higher magnification of the normal brain (H/E x400).

Figure 5: Group E: Normal Brain at a Higher

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Magnification (H/E x400) Normal neurons (thick arrow) with normochromic nucleus and pale cytoplasm are present, as are normal glial cells (thin arrow). Normal fibrillary extensions are found in the background.

DISCUSSION

Key components of the brain include the cerebral cortex, which forms memories, homeostasis, information processing, motor control, arousal, and perception. Additionally, it incorporates general movement, visceral functions, behavioral reactions, and higher mental functions [20–21].

In this study, we looked into how molly affected the adult Wistar rats' brains histomorphologically. We found no evidence of brain cell damage in teenage rats at a level that does not cause serotonergic neurotoxicity. The main conclusions were: 1) Normal neurons with a normochromic nucleus and pale cytoplasm are present; 2) Normal glial cells are also present. There are typical fibrillary extensions in the backdrop. Since human studies revealed that "ecstasy" dance clubbers had body temperature increases of approximately 1 °C [22], our dosing scheme more nearly replicates the human condition, making the extrapolation to human adolescents more credible.

Since MDMA misuse causes several physiological changes, we also assessed food and water intake in addition to body weight growth. The anorectic effects and cardiovascular alterations of amphetamines and MDMA have been extensively documented in the literature [3]. Both groups of animals had comparable levels of body weight loss, which may have been caused by stress brought on by touching the animals during the experiment. However, in the 24 hours after treatment, water intake in rats treated with MDMA increased somewhat, most likely as a result of the drug's ability to cause hyperthermia. Unlike procedures that expose animals to MDMA over days, our acute protocol did not cause reduced weight gain or animal dehydration [23]. Consequently, it would seem that dehydration has little bearing on our overall findings.

effects in lab animals [24–25].

One key contributor to MDMA's toxicity is its metabolism, which leads to the formation of highly reactive metabolites capable of inducing oxidative stress [4]. Studies have shown that MDMA metabolites, such as α -methyldopamine (α -MeDA, 3,4-dihydroxyamphetamine, HHA) and N-methyl- α -methyldopamine (NMe- α -MeDA, 3,4-

dihydroxymethamphetamine, HHMA), can cause neuronal death in cultured cells [26–27]. These metabolites, particularly α -MeDA and NMe- α -MeDA, can undergo oxidation to form orthoquinones. This process initiates a redox cycle that generates oxidative stress [4].

Furthermore, thioether metabolites of MDMA have been found to promote the formation of quinoproteins and deplete glutathione (GSH) in cultured neurons, exacerbating oxidative stress [28]. Research also indicates that MDMA and its metabolites disrupt mitochondrial dynamics, including fusion/fission balance and intracellular trafficking, in cultured neurons [29]. In vitro studies have demonstrated that catechol metabolites of MDMA increase toxicity to both hepatocytes and cardiomyocytes [30]. Other mechanisms contributing to MDMA-induced oxidative stress include the metabolism of monoamine neurotransmitters by monoamine oxidase [2] and the production of nitric oxide, which generates harmful reactive nitrogen species [4].

In our study, no oxidative stress-related changes were detected in the adolescent rat brain under the selected MDMA exposure paradigm. However, as noted in Table 1, there was a significant reduction in body weight during and after MDMA treatment. The exact mechanism underlying the observed MDMA-induced cellular degeneration remains unclear and warrants further investigation.

To address the potential limitations of this study, future research should explore the effects of chronic MDMA exposure and higher doses to determine whether these conditions might induce oxidative stress-related changes and contribute to neurodegeneration.

CONCLUSION

In conclusion, modest dosages of MDMA, which are more in line with what teenagers in real life consume, do not cause oxidative stress-related brain alterations in adolescent rats. Adult rats treated with MDMA did not exhibit changes in brain morphology or histology. To determine whether moderate MDMA dosages cause brain alterations in adult animals and whether these changes are permanent and might manifest later in maturity or old age, more research is needed. According to our findings, adult

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Wistar rats' brain histology does not exhibit neurodegenerative effects from molly exposure.

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APPENDIX

Table 1: Body Weight Changes of Rats Fed with Graded Dose of Molly at Various Intervals

W eig ht (g)	A Con trol (n= 5)	B (0.12 mg/k g) (n=5)	C (0.1 8mg/ kg) (n=5)	D (0.3 2mg/ kg) (n=5)	E (0.4 mg/ kg) (n= 5)	F v al u e	P va lu e
W	252	277.	252.	272.	215.	2	0.
B	.5±	5±3.	5±3.	5±3.	5±2.	9.	00
M	3.5	53	53	53	12	3	1
A	3					0	(S)
W	327	310.	273.	295.	277.	1	0.
A	.5 ±	0±14	0±2.	0±7.	0±3.	8.	00
M	3.5	.14	32	07	53	1	4
A	3					8	(S)

KEY:

P-value (p<0.05): significant

WBMA: weight before MDMA administration

WAMA: weight after MDMA administration

Values are mean ± standard deviation

n: Number of sample

s: significant

n/s: not significant

Histomorphological Changes

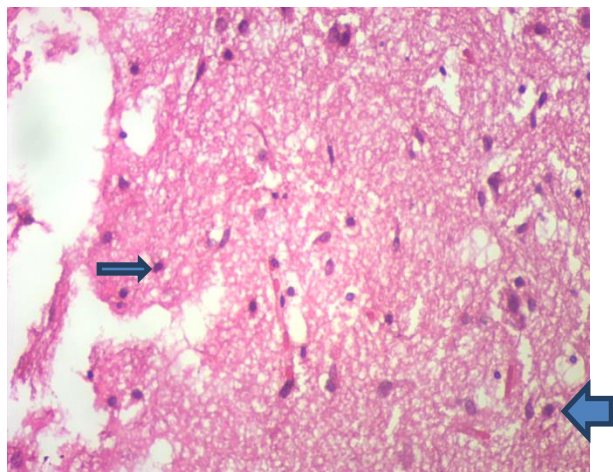


Figure 1: Control-Group A- Section of the brain shows the presence of normal neurons (thick arrow) with normochromic nucleus and pale cytoplasm. Also present are normal glial cells (thin arrow). On the background are normal fibrillary extensions. Normal Brain at a higher magnification (H/E x400).

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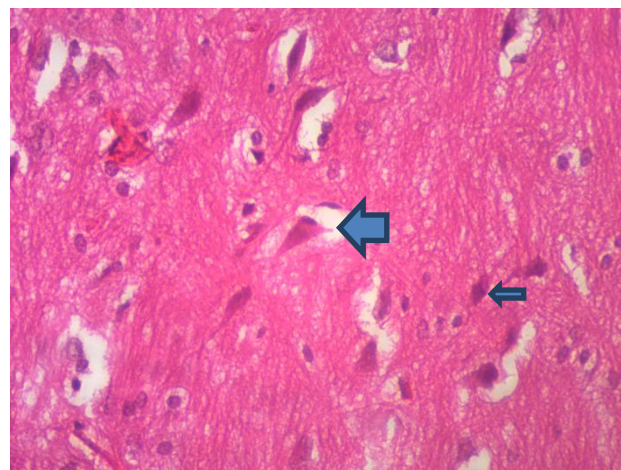


Figure 3: Group C- Section of the brain shows the presence of normal neurons (thick arrow) with normochromic nucleus and pale cytoplasm. Also present are normal glial cells (thin arrow). On the background are normal fibrillary extensions. Normal Brain at a higher magnification (H/E x400)

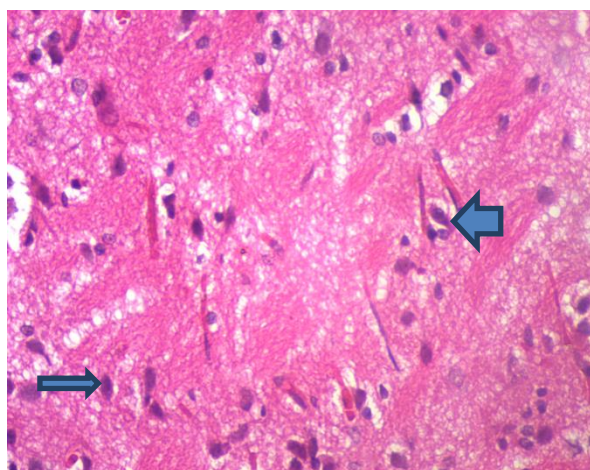


Figure 2: Group B- Section of the brain shows presence of normal neurons (thick arrow) with normochromic nucleus and pale cytoplasm. Also present are normal glial cells (thin arrow). On the background are normal fibrillary extensions. Features are in keeping with normal brain tissue. Normal Brain at higher magnification (H/E x400).

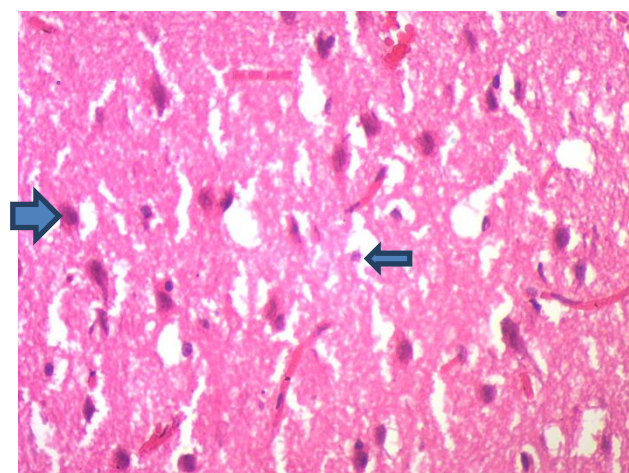


Figure 4: Group D- Section of the brain shows presence of normal neurons (thick arrow) with normochromic nucleus and pale cytoplasm. Also present are normal glial cells (thin arrow). On the background are normal fibrillary extensions. Normal Brain at a higher magnification (H/E x400)

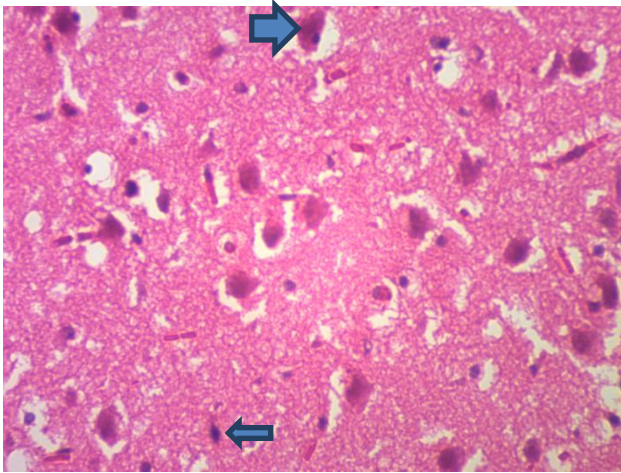


Figure 5: Group E- Section of the brain shows presence of normal neurons (thick arrow) with normochromic nucleus and pale cytoplasm. Also present are normal glial cells (thin arrow). On the background are normal fibrillary extensions. Normal Brain at a higher magnification (H/E x400)