Effect of paracetamol on the post-natal development of the medial prefrontal cortex in albino rat and the potential protective role of silymarin

Merry B.K. Shenouda[a*] and Omnia I. Ismail[a]

[a]Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Assiut, Egypt

Abstract

Background: One of the popular painkillers, paracetamol is thought to be safe for use in pregnant women, and nursing mothers.

Objectives: To observe the effect of paracetamol on the development of the medial prefrontal cortex (mPFC) in albino rats and to evaluate the protective effect of silymarin.

Material and Methods: Forty pregnant rats were divided randomly into four equal groups: Group A: received nothing. Group B: was given silymarin at a dose of 200 mg/kg body weight orally once daily. Group C: was given paracetamol at a dose of 350 mg/kg body weight orally once dailly. Group D: was given paracetamol and silymarin. Treatment started at the sixth day of the gestation until the end of lactation. After weaning, the pups received the same regimen until the age of three months. The offspring were selected from each group at the following ages: 1 day, 21 days and 3 months The mPFC was processed for light, ultrastructural and immunohistochemical analyses.

Results: Paracetamol exposure led to hypocellularity in different layers of mPFC, shrunken pyramidal neurons, vacuolated neuropil, weak SYN immunoreaction and a decrease in the number of pyramidal cells and thickness of the mPFC. Silymarin coadministration induced a partial restoration of normal arrangement of the mPFC, the cytoarchitecture of pyramidal neurons, SYN immunoreaction as well as an increase in the number of the pyramidal cells and thickness of the mPFC.

Conclusion: Paracetamol treatment caused neuronal damage in the mPFC. Silymarin appeared to be beneficial in protecting mPFC' structure.

Keywords: Paracetamol; Silymarin; Synaptophysin; Medial prefrontal cortex; Development.

DOI: **10. 21608/svuijm.2023.195676.1538**

*Correspondence: merrybeniamen@aun.edu.eg

Received: 1 February,2023.

Revised: 20 February,2023.

Accepted: 25 February,2023.

Published: 15 March, 2023

Cite this article as: Merry B.K. Shenouda and Omnia I. Ismail (2023). Effect of paracetamol on the post-natal development of the medial prefrontal cortex in albino rat and the potential protective role of silymarin *SVU-International Journal of Medical Sciences*. Vol.6, Issue 2, pp: 10-36. .

Introduction

Analgesia is frequently used during pregnancy, the newborn stage, and childhood. However, little is known about their safety because users are customarily excluded from clinical research **(Philippot et al., 2017).**

One of the most popular painkillers, paracetamol (acetaminophen, N-acetyl-p-aminophenol) is thought to be safe for use in babies, pregnant women, and nursing mothers. It is uniformly distributed throughout the central nervous system and can penetrate the blood-brain barrier at therapeutic and lethal dosages **(Karakilic et al., 2022).**

On the other hand, there is abundant evidence that paracetamol administration during pregnancy has been linked to IQ decline, language delay, and attention deficit hyperactivity disorder (ADHD) or autism spectrum disorders (ASD) **(Hussin & Al-Allaf, 2022)**.In addition, researchers found that postnatal acetaminophen exposure may be associated with an elevated incidence of ASD **(Alemany et al., 2021).**Contrarily, there are limited data considering postnatal exposure to paracetamol, and it is difficult to address the problem using a traditional multivariate analysis of epidemiologic data due to several variables **(Patel et al., 2022).**

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder that lasts into adulthood and affects 2.5 percent of adults worldwide, ADHD is characterized by clinical features of hyperactivity, inattention, and impulsivity **(Song et al., 2021).**

The prefrontal cortex (PFC) can be divided into three major areas based on connectivity: the medial PFC (the cingulate area 2, prelimbic, infralimbic, and medial and ventral orbital regions), motor PFC (the premotor and cingulate area 1 regions), and orbital PFC (the anterior insular and lateral orbital regions) **(Yang et al., 2022).**

Planning and numerous cognitive processes such as one's own location within the environment, context discrimination and the recognition of novelty/familiarity in the surrounding characteristics depend on the mPFC **(Sauer et al., 2022)**. In addition, the mPFC responds also to visual stimuli by developing a visuomotor link **(Peters et al., 2022).**

Furthermore, through its connections with the basal ganglia (the ventral striatum and ventral tegmental area), the mPFC has been implicated in the executive regulation of goal-directed behavior. Dysregulation of the mPFC has often been linked to disorders of goal-directed behavior such as depression and drug addiction **(Beier et al., 2015).**

Many studies have concluded that mPFC damage in newborns induces an impairment in executive functioning, disturbed motivating, and emotional behaviors, and increases the risk of ADHD **(Zararsız et al., 2004; Watanabe, 2016 and Miguel et al., 2018).**

The brain is incredibly vulnerable to reactive oxygen species and oxidative stress due to its high concentrations of polyunsaturated fatty acids and relatively low quantity of antioxidative enzymes as well as high oxygen consumption rate of the brain

cells. Silymarin, a flavonolignan derived mostly from the seeds of milk thistle (Silybum marianum), is a combination of silybin, silidianin, and silychristine, with silybin being the most active **(El-Din et al., 2014).**

Silymarin is primarily eliminated by the bile and, to a lesser extent, through the urine. In the liver, silibinin and other silymarin components are quickly conjugated with glucuronic acid and sulphate then flow into the plasma and the bile. The intestinal flora hydrolyzes it and reabsorbs it in the gut. Insignificant amounts are excreted in urine **(Khazaei et al., 2022).**

It has a vast range of pharmacological effects, including anti-inflammatory, anti-proliferative, antioxidant, and anti-angiogenic actions. It has been well known that silymarin has been utilized as a medication to treat cirrhosis, alcoholic liver disease, acute and chronic viral hepatitis, and toxin/drug-induced hepatitis **(Adelina, 2022)**. Also, it can cease the peroxidation processes that lead to liver lesions elicited by paracetamol, halothane, carbon tetrachloride, ethanol, phalloidin, galactosamine and other poisonous drugs **(Khazaei et al., 2022).**

Several internal organs have been subject to silymarin's tissue-protecting effects such as the kidney **(Senturk et al., 2008),** heart **(Rao & Viswanath, 2007)** , hippocampus **(Hirayama et al., 2016)** and neurons of the substantia nigra pars compacta **(Raza et al., 2011)**. Moreover, it likely improves memory and learning impairments because of its antioxidant effects **(Bahram et al., 2020).**

To our knowledge, toxicity associated with paracetamol treatment in the brain is poorly investigated and there is no experimental study concerning the protective effects of silymarin against neuronal damage in the mPFC generated by paracetamol. Therefore, the aim of this study was to observe the effect of paracetamol on the development of the mPFC and to evaluate the protective effect of concomitant treatment with silymarin.

Materials and methods

Chemicals

Paracetamol and silymarin were purchased from Sigma–Aldrich, St. Louis, MO, USA.

Animals

Forty female albino rats and twenty male albino rats aged three months and weighted 200 -220 gm were bought from the Animal House, Faculty of Medicine, Assiut University. The female rats were isolated for one week to be sure that they were non pregnant. They became pregnant after overnight mating with adult male albino rats (one male for two female rats). By light microscopic examination, detection of the sperms in the vaginal plug was recorded as 0-day pregnancy **(Mahmoud, 2001)**.

The rats were housed in airy broad cages under normal laboratory conditions following the Guidelines on Care and Use of Laboratory Animals **(Essiet et al., 2017).** All methods were performed in accordance with the relevant guidelines and regulations and compliance with ARRIVE guidelines for the care and use of experimental animals by the Committee for the Supervision of Experiment on Animals (CPCSEA) and the National Institute of Health Protocol **(Parasuraman et al., 2015)**. Animal manipulation through the study was approved by the ethics committee (IRB local approval

number: 17300855) at the Faculty of Medicine, Assiut University, Assiut, Egypt.

Experimental design

Forty pregnant rats were divided randomly into four equal groups:

Group A (negative control): 10 pregnant rats did not receive any treatment.

Group B (positive control): 10 pregnant rats were given silymarin at a dose of 200 mg/kg body weight once daily orally by a gastric tube. The selected dose was chosen according to its efficacy to show the maximal protection in variant types of brain diseases **(Lu et al., 2009).**

Group C (paracetamol treated): 10 pregnant rats were orally given paracetamol at a dose of 350 mg/kg by gastric tube. The dosage of paracetamol is a dosimetric adjustment of 500 mg/day for humans (65 kg) plus an additional security factor of 10 to account for intraspecies variability **(Yang et al., 2013).**

Group D (paracetamol+ silymarin treated): 10 pregnant rats were given paracetamol and silymarin at the same previously stated doses.

The offspring were selected from each group of the pregnant rats at the following ages:

- 1 day (Subgroup I A, I B, I C and ID): each subgroup contained 10 animals.
- 21 days (Subgroup II A, II B, II C and II D): each subgroup contained 10 animals.
- 3 months (Subgroup III A, III B, III C and III D): each subgroup contained 10 animals.

The treatment to pregnant rats began on the sixth day of the gestation and continued until birth, as well as during the lactation period until weaning. After weaning, the pups were separated from their mothers and divided into four subgroups (group III A, III B, III C and III D). They received the same regimen as the pregnant rats until the age of three months.

At the end of the experiment, animals from each subgroup were given an intraperitoneal injection of 50 mg/kg pentobarbital to induce anesthesia. The two cerebral hemispheres of each rat were made visible by removing the skull's vault. The complete brains were delicately dissected, swiftly taken from the skulls and kept on an ice-cold plate.

Histological procedures

A) Light microscopic study

To harden and prevent brain tissue dissipation, two harvested brains from each group were fixed in a 10% neutral buffered formalin solution. Brains were implanted in blocks of paraffin after standard procedures. Cresyl fast violet was used to stain coronal paraffin sections (5 μm thick) cut at the level of the mPFC.

B) Transmission electron microscopic study

The brain samples from mPFC were cut into tiny pieces (1 mm3) with a fine glass knife, fixed in 2.5 percent phosphate buffered glutaraldehyde and processed for the semithin sections stained with toluidine blue stain **(Kuo, 2007**).

The transmission electron microscope (TEM) ("Jeol" E.M.-100 CX11; Japan) at the Electron Microscopic Unit of Assiut University, Assiut- Egypt was used to analyze the ultrathin slices stained with lead citrate and uranyl acetate.

C) Immunohistochemical study

Paraffin sections were moistened, deparaffinized in xylene, and then immersed in phosphate-buffered saline (PBS; pH 7.6). The samples were

boiled in citrate buffer (0.01 M) for 15 minutes to remove the antigens. Sections were treated for 5 minutes with 3 percent hydrogen peroxide to stop endogenous peroxidase activity, then rinsed with deionized water and PBS. To reduce non-specific staining, sections were first treated with 1 percent pre-immune rabbit serum. Thereafter, a monoclonal antibody against synaptophysin (SYN) protein (Thermo Fisher Scientific, Massachusetts, USA) with a dilution of 1:20 was added overnight at 4 °C. Using a biotin-streptavidin detection technique with 3,3'-Diaminobenzidine (DAB) (Sigma Aldrich, USA) as a chromogen, the primary antibodies were detected. Sections underwent dehydration, Mayer's hematoxylin counterstaining, and finally permount cover-slipping. In SYN-positive neurons, the surface of the neuronal cell bodies and their processes were covered in brown beaded granules as described by **(Adedayo et al., 2017).**

D) Morphometric and statistical analysis

Using the image J software (ImageJ v1.51a), the pyramidal cells were counted in the internal pyramidal layer zone and the area percent of SYN immunoreaction was assessed in 10 distinct fields at a magnification of 400/slide from ten individual rats for each group. The mPFC's thickness was measured as a Line drawn from the mPFC's surface to the transition between the grey and white matter at a magnification of 100 in 10 distinct fields.

GraphPad Prism 8.3.8 (GraphPad Software Inc., San Diego, CA, USA) was used for the statistical analysis of all data, which is presented as mean ±standard deviation (SD) of the mean. One-way analysis of variance (ANOVA) was employed to analyze differences between the groups, followed by post hoc Tukey's test. When the p-value was ≤ 0.05, the differences between the groups were thought to be statistically significant.

Results

Examination of the sections in the negative and positive control groups showed a normal histological appearance of mPFC in all age groups, so the control group referred to both.

One-day-old age groups:

A) Light microscopic study

Examination of the cresyl fast violet-stained sections in the control group showed that the mPFC consisted of the following layers: the molecular, external granular, external pyramidal, inner granular, inner pyramidal and polymorphic layer. The cells appeared dense, numerous, immature, and smaller than those in other age groups. There were also pyramidal cells with triangular soma and deeply stained Nissl substance as well as rounded small granular cells (**Fig.1a**).

When compared to the control group, the paracetamol-treated group appeared hypocellular in different layers of mPFC and lightly stained pyramidal cells, but normal granular cells were still present (**Fig.1b**).

The paracetamol+ silymarin treated group restored of the normal arrangement of the mPFC layers seen in the control group. The pyramidal cells and the granular cells had deeply stained Nissl granules more or less similar to the control group **(Fig.1c**).

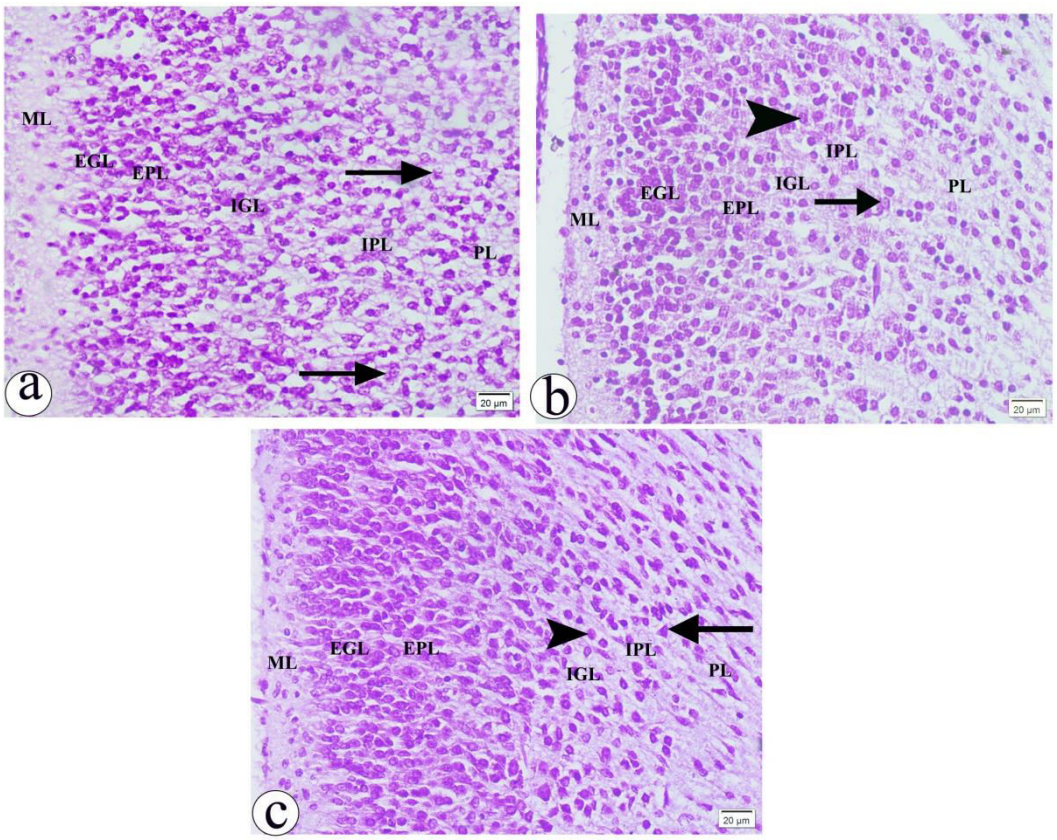

Fig. 1. The photomicrographs of the sections in the medial prefrontal cortex of 1-day-old rats in (a) The control group showing that mPFC consisted of the following layers: the molecular layer (ML), the external granular layer (EGL), the external pyramidal layer (EPL), the internal granular layer (IGL), the internal pyramidal layer (IPL) and the polymorphic layer (PL). The cells appear dense, numerous, immature, and smaller than those of other age groups. The pyramidal cell (arrow) with triangular soma and deeply stained Nissl substance as well as deeply stained rounded granular cells (arrow- head) are observed. (b) The paracetamol treated group showing apparent hypocellularity in different layers of mPFC. Lightly stained pyramidal cell (arrow) and normally appeared granular cells (arrow- head) are seen. (c) The paracetamol+ silymarin treated group showing restoration of the normal arrangement of the layers of mPFC. The pyramidal cells (arrow) and the granular cells (arrowhead) have deeply stained Nissl granules that are more or less similar to the control group. (Cresyl fast violet stain, x400, scale bar = 20 µm)

In control sections stained with toluidine blue the pyramidal cells had vesicular nuclei framed by cytoplasm having basophilic Nissl's granules with intact apical dendrites. The granular cells and the microglial cells with dark nuclei appeared normal. Also, intact neuropil is seen (**Fig. 2a**). In contrast, paracetamol treated group showed some dense cells surrounded by wide perineuronal space and perineural glial cells. Vacuolated cytoplasm in many cells and vacuolated neuropil were observed (**Fig. 2b**). Some normal-appearing pyramidal cells with vesicular nuclei and intact apical dendrites were found in the paracetamol+silymarin-treated group. Other pyramidal cells were shrunken with irregular outline. Some vacuolations appeared in the neuropil (**Fig. 2c**).

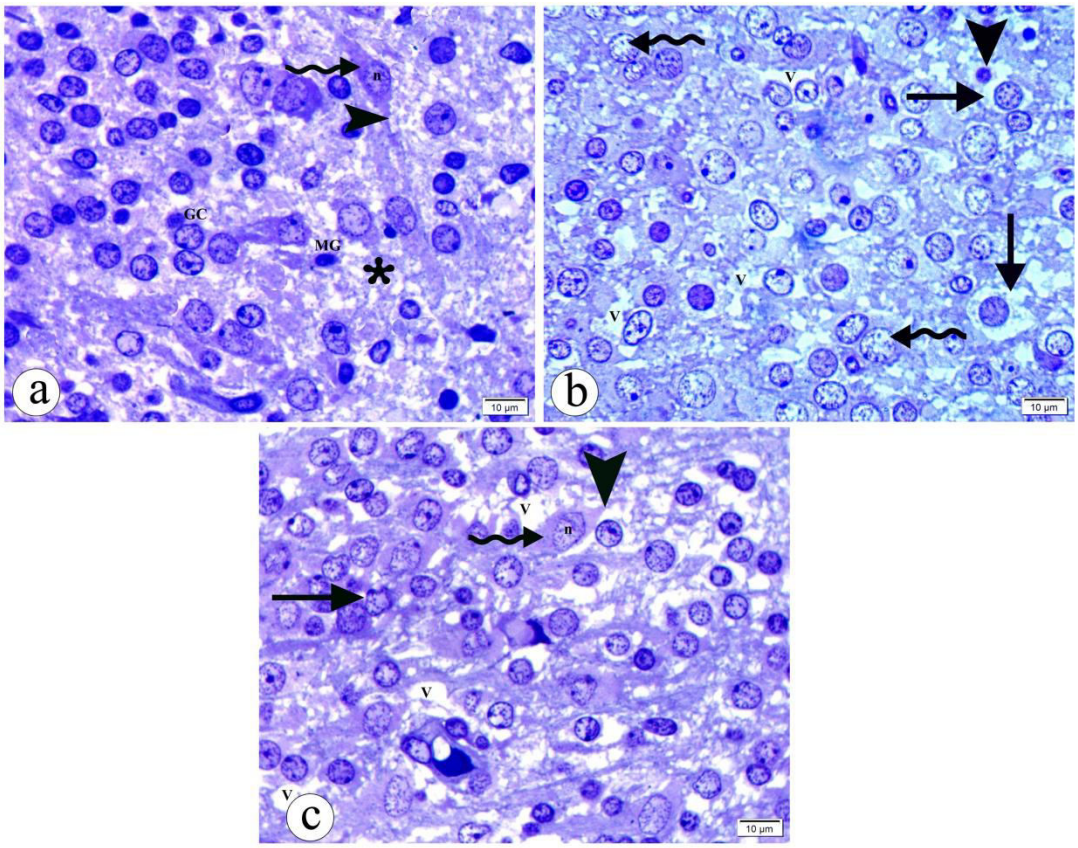

Fig. 2. The photomicrographs of the semithin sections in the medial prefrontal cortex of the 1-day-old rats in (a) The control group showing the pyramidal neurons with vesicular nuclei (n) framed by cytoplasm (curved arrow) that having basophilic Nissl's granules with intact apical dendrites (arrowhead). The granular cells (GC) and the microglial cells (MG) with dark nuclei appeared normal. The intact neuropil (asterisk) is seen. (b) The paracetamol treated group showing some dense neurons surrounded by wide perineuronal space (arrow) and perineural glial cells (arrowhead). Vacuolated cytoplasm (curved arrow) in many neurons and vacuolated neuropil (V) are observed. (c) The paracetamol+ silymarin treated group showing normal appeared pyramidal cells with vesicular nuclei (n) framed by cytoplasm (curved arrow) and basophilic Nissl's granules with intact apical dendrites (arrowhead). Other pyramidal cells (arrow) are shrunken with an irregular outline. Some vacuolations (V) appear in the neuropil. (Toluidine blue x1000, Scale bar= 10 µm)

B) Electron microscopic study

The electron microscopic evaluation in the control group displayed pyramidal cells with euchromatic nuclei. The cytoplasm showed normal cellular organelles like mitochondria, rough endoplasmic reticulum, and ribosomes (**Fig. 3a**).

In the paracetamol treated group, the pyramidal cells had nuclei with chromatolysis. Rarefied cytoplasm was observed. Destructed mitochondria dilated rough endoplasmic reticulum and many vacuolations in the cytoplasm were noticed. Additionally, cellular infiltration was observed (**Fig. 3b**). Regarding the paracetamol+ silymarin treated group, the pyramidal cells had normal euchromatic nuclei, but loss of the cell organelles was still present (**Fig. 3c**).

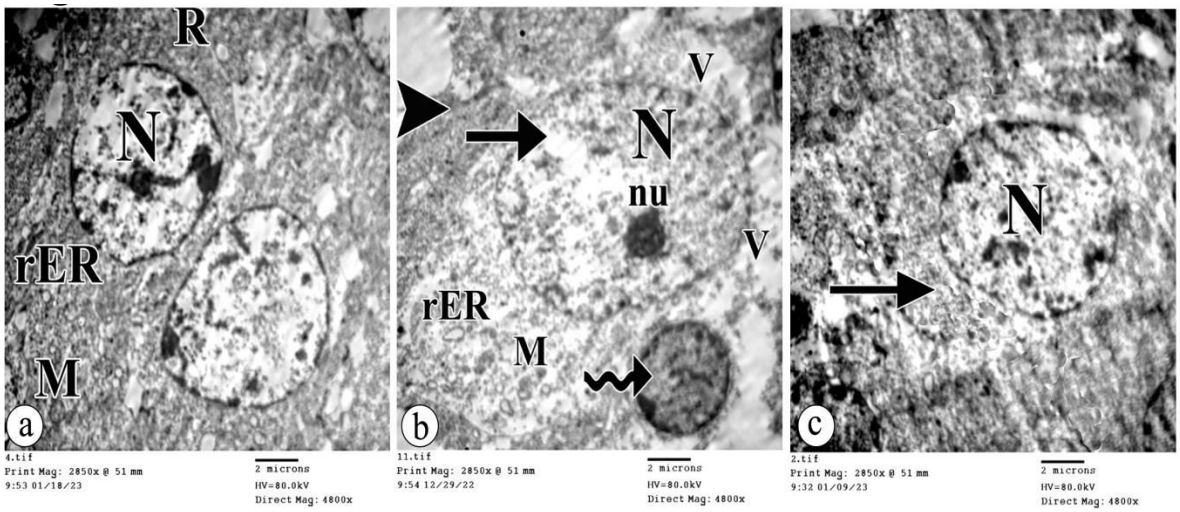

Fig.3. The electron photomicrographs of the pyramidal cell of 1-day-old rats in (a) the control group showing a pyramidal cell having euchromatic nucleus (N). The cytoplasm shows mitochondria (M), rough endoplasmic reticulum (rER) and ribosomes (R). (b) The paracetamol treated group showing nucleus (N) with chromatolysis (arrow), nucleolus (nu), rarefied cytoplasm (arrowhead), destructed mitochondria (M), dilated rough endoplasmic nucleus (rER), and many vacuolations (V). The cellular infiltration (curved arrow) is observed. (c) The paracetamol+ silymarin treated group showing a pyramidal cell having euchromatic nucleus (N). Loss of cell organelles is also observed. (TEM x4800, Scale bar= 2 μm)

C) Immunohistochemical results

Regarding immunohistochemically stained sections for SYN, the control group showed an under-developed moderate reaction on the surface of normal cells (**Fig.4a**). In comparison, the paracetamol treated group showed a weak SYN reaction on the surface of affected cells (**Fig. 4b**). Interestingly, the paracetamol+ silymarin treated group exhibited a moderate SYN reaction on the surface of normal cells (**Fig. 4c**).

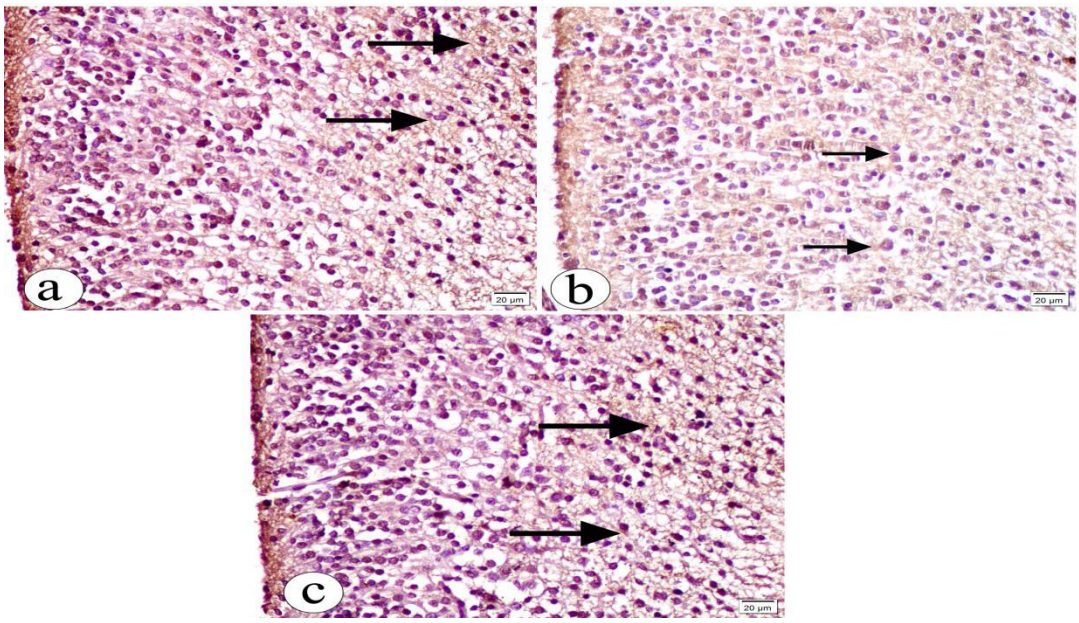

Fig. 4. The photomicrographs of the sections in the medial prefrontal cortex of 1-day -old rats in (a) The control group showing a mild SYN reaction (arrow) on the surface of normal cells. (b) The

paracetamol treated group showing a weak SYN reaction (arrow) on the surface of affected cells. (c) The paracetamol+ silymarin treated group showing a moderate SYN reaction (arrow) on the surface of normal cells. (SYN immunostaining, x400, scale bar = 20 μm).

21-day-old age groups

A) Light microscopic study

Examination of cresyl violet-stained sections in the control group revealed the normal appearance of different layers of the mPFC (**Fig.5a**). Additionally, deeply stained Nissl pyramidal cells and granular cells were detected (**Fig. 5b**).

The paracetamol treated group, on the other hand, showed an apparently hypocellularity in different layers of mPFC (**Fig. 5c**). Moreover, the paracetamol treated group demonstrated dense shrunken pyramidal cells, poorly stained vacuolated neuropil, cellular infiltration, and dilated blood vessels. Some pyramidal cells appeared shrunken surrounded by a wide perineuronal space and glial cells. Moreover, other pyramidal cells showed lightly stained Nissl granules. Some granular cells had loss of Nissl granules. Some microglial cells appeared with dilated perineuronal spaces (**Fig. 5d**).

The paracetamol+ silymarin treated group showed a restoration of the layers of mPFC (**Fig.5e**). In addition, normal blood vessels and microglial cells were seen. Some granular cells appeared to have moderately stained Nissl granules, but others showed loss of Nissl granules. The pyramidal cells had vesicular nuclei with peripheral process and moderately stained Nissl granules but others had lightly stained Nissl granules (**Fig.5 f**).

Examination of toluidine blue-stained sections in the control group revealed that normal appearing pyramidal cells, intact processes, the microglial cells and normal blood vessel within the intact neuropil were observed (**Fig. 6a**).

In contrast, the paracetamol-treated group demonstrated shrunken dense pyramidal cells with an irregular outline surrounded by haloes. Some pyramidal cells had deeply stained nuclei and dense cytoplasm. However, other pyramidal cells still appeared normal. In addition, some microglial cells surrounded by dilated perineuronal space and dilated blood vessels in intact neuropil were noticed (**Fig. 6b**).

While paracetamol+ silymarin treated group revealed some pyramidal neurons with vesicular nuclei framed by dense cytoplasm. Other pyramidal neurons appeared shrunken with an irregular outline and dilated perineuronal space. Some intact processes and dilated congested blood vessel were observed (**Fig. 6c**).

B) Electron microscopic study

The electron microscopic examination showed that the pyramidal cells in the control group had a normal histological appearance (**Fig. 7a**).

In the paracetamol treated group, the pyramidal cells showed heterochromatic shrunken nuclei. The cytoplasm displayed loss of the cell organelles and many vacuolations (**Fig. 7b**).

Interestingly, the paracetamol+ silymarin treated group showed pyramidal cells with euchromatic nuclei and prominent nucleoli. The cytoplasm showed normal-appearing cell organelles more or less like the control (**Fig. 7c**).

C) Immunohistochemical study

Immunohistochemical staining for SYN demonstrated that the control

group had a strong reaction on the surface of normal pyramidal cells (**Fig. 8a**). On the other hand, the paracetamol treated group revealed a faint weak SYN reaction on the surface of affected pyramidal cells (**Fig. 8b**). Moderate SYN reaction on the surface of normal pyramidal cells was appeared in the paracetamol+ silymarin treated group (**Fig. 8c**).

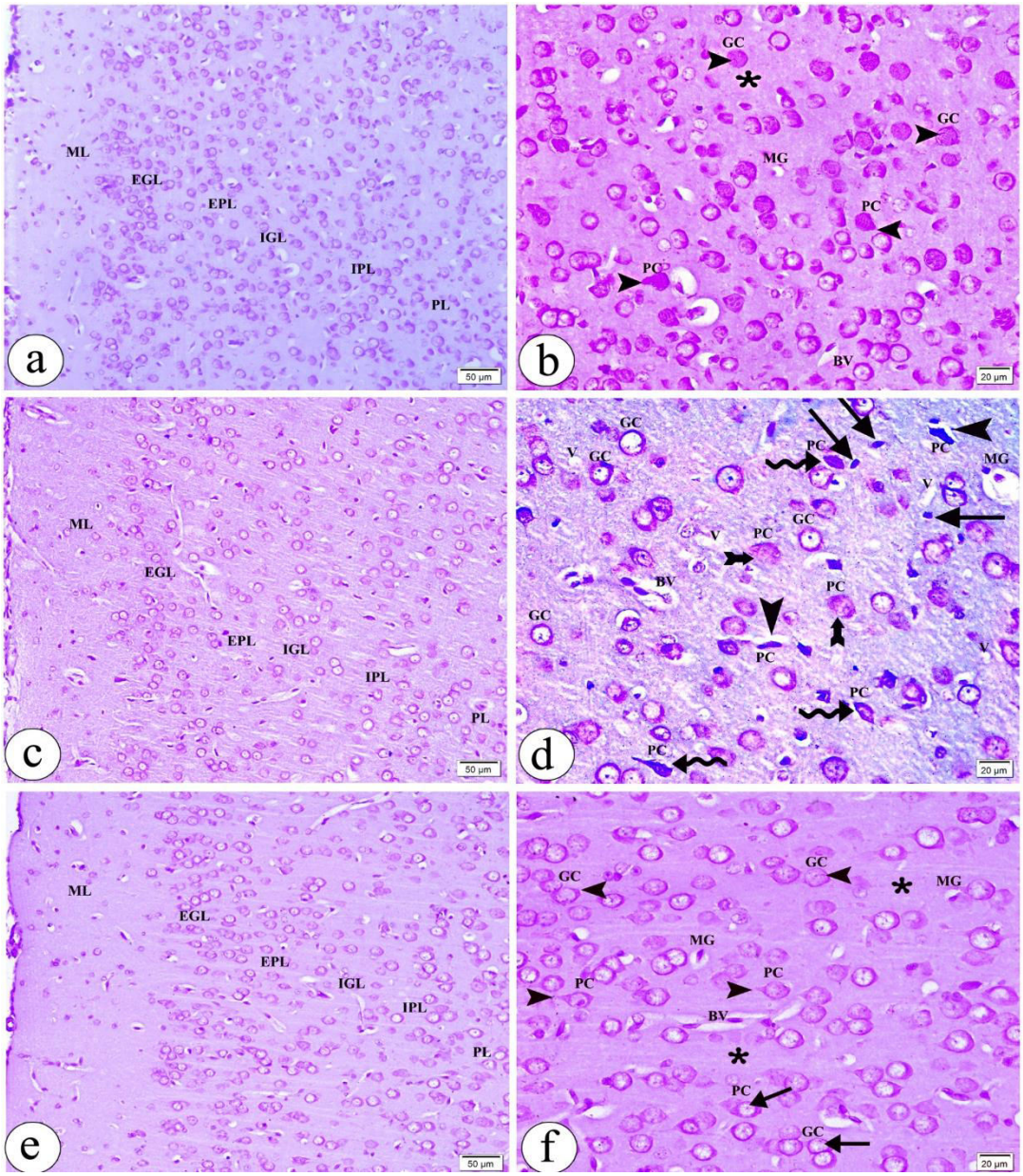

Fig. 5. The photomicrographs of the sections in the medial prefrontal cortex of 21-day-old rats in (a) The control group showing the layers of the mPFC: the molecular layer (ML), the external granular layer (EGL), the external pyramidal layer (EPL), the internal granular layer (IGL), the internal pyramidal layer (IPL) and the polymorphic layer (PL). (b) The control group showing deeply stained Nissl granules (arrow-head) in the pyramidal cells (PC) and granular cells (GC). Notice normal blood vessel (BV), intact neuropil (asterisk) and microglial cells (MG). (c) The paracetamol treated group showing apparent hypocellularity in different layers of mPFC. (d) The paracetamol treated group showing dense shrunken pyramidal cells (curved arrow), poorly stained vacuolated neuropil (V), cellular infiltration (arrow) and dilated blood vessels (BV). Some pyramidal cells (PC) appear dense and shrunken and surrounded by wide perineuronal space (arrow-head). Other pyramidal cells (PC) show lightly stained Nissl granules (tailed arrow). Some granular cells (GC) show a loss of Nissl

granules. Some microglial cells (Mg) are surrounded by dilated perineuronal space. (e) The paracetamol+ silymarin treated group showing restoration of the layers of mPFC. (f) The paracetamol+ silymarin treated group showing that the blood vessels (BV) and microglial cells (MG) appear normal. Some granular cells (GC) have moderately stained Nissl granules (arrowhead). Other granular cells (GC) show loss of Nissl granules (arrow). The pyramidal cells (PC) have a vesicular nucleus with a peripheral process (arrowhead) and moderately stained Nissl granules. Some pyramidal cells (PC) show lightly stained Nissl granules (arrow). Notice the deeply stained neuropil (asterisk). (Cresyl fast violet stain, a, c, e X200, scale bar = 50 µm & b, d, f x400, scale bar = 20 µm)

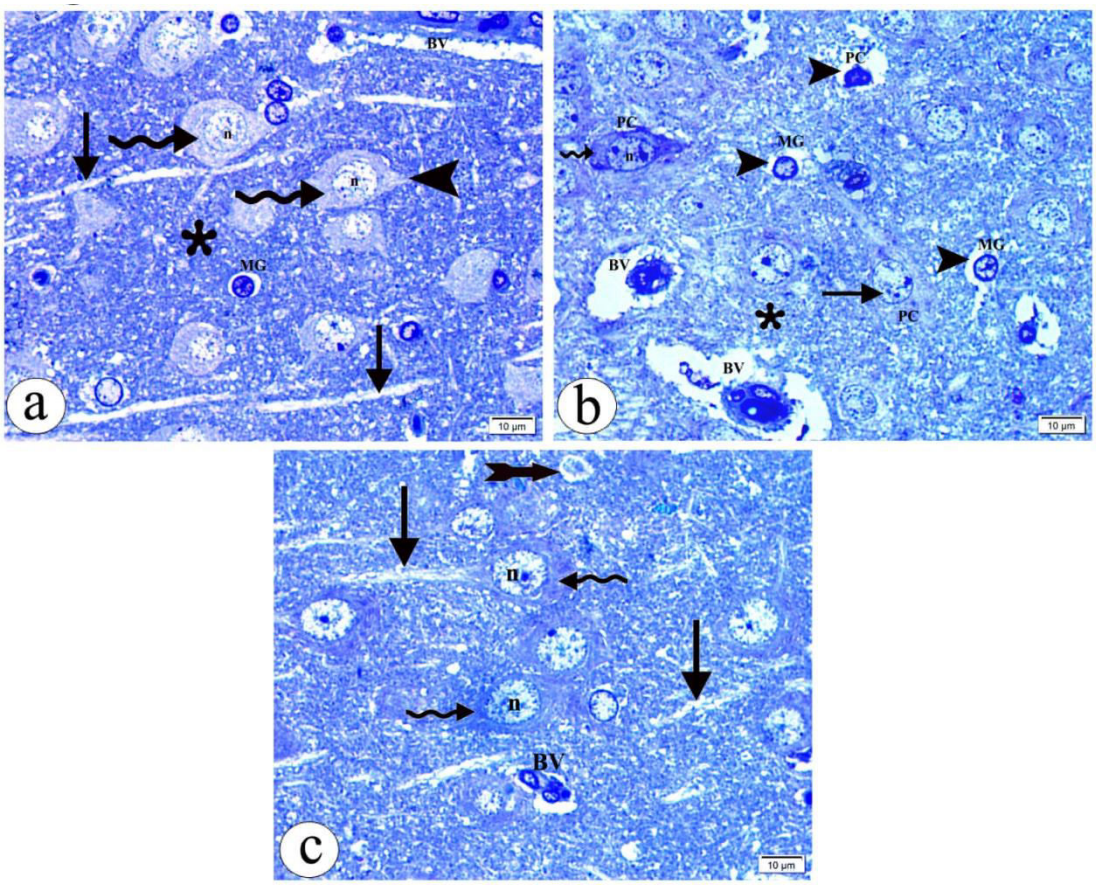

Fig. 6. The photomicrographs of the semithin sections in the medial prefrontal cortex of the 21-day-old rats in (a) The control group showing the pyramidal neurons with vesicular nuclei (n) framed by pale cytoplasm (curved arrow) that have basophilic Nissl's granules with intact apical dendrites (arrowhead). Some intact processes (arrow) and the microglial cells (MG) can be observed. Normal blood vessel (BV) within the intact neuropil (asterisk) are seen. (b) The paracetamol treated group showing shrunken dense pyramidal neurons (PC) with irregular outline and surrounded by haloes (arrowhead). Some pyramidal neurons (PC) show deeply stained nuclei (n) and dense cytoplasm (curved arrow). Other pyramidal neurons (PC) appear normal (arrow). Some microglial cells (MG) show dilated perineuronal space (arrow-head). Dilated blood vessels (BV) in intact neuropil (asterisk) can be observed. (c) The paracetamol+ silymarin treated group showing some pyramidal neurons with vesicular nuclei (n) framed by dense cytoplasm (curved arrow). Other pyramidal neurons appear shrunken with irregular an outline and dilated perineuronal space (tailed arrow). Some intact processes (arrow) and dilated congested blood vessels (BV) can be observed. (Toluidine blue x1000, Scale bar= 10 µm)

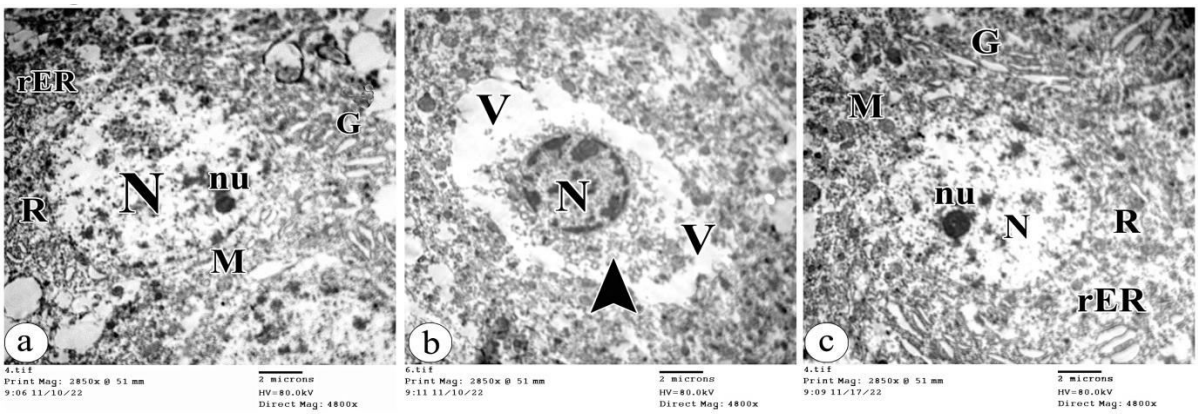

Fig. 7. The electron photomicrographs of the pyramidal cell in 21-day-old rats in (a) The control group showing a pyramidal cell having euchromatic nucleus (N) with a prominent nucleolus(nu).The cytoplasm shows well developed Golgi apparatus (G), mitochondria (M) , rough endoplasmic reticulum (rER) and free ribosomes (R). (b) The paracetamol treated group showing a pyramidal cell with heterochromatic shrunken nucleus (N). The cytoplasm displays loss of the cell organelles (arrow-head) and many vacuolations (V) are noticed. (c) The paracetamol+ silymarin treated group showing a normal pyramidal cell with euchromatic nucleus (N) and a prominent nucleolus (nu). The cytoplasm shows a well-developed Golgi apparatus (G), rough endoplasmic reticulum (rER), many mitochondria (M) and free ribosomes (R). (TEM x4800, Scale bar= 2 µm)

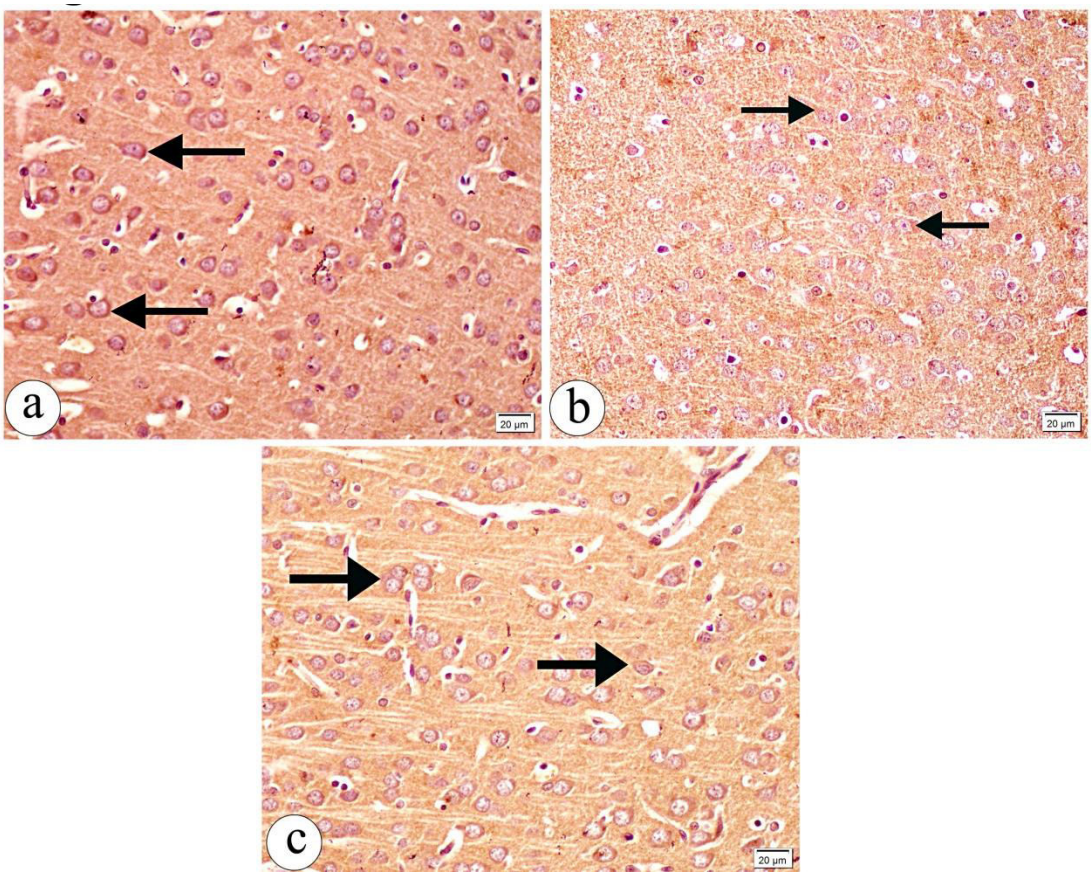

Fig. 8. The photomicrographs of the sections in the medial prefrontal cortex of 21-day old rats in (a) The control group showing a dense and strong SYN reaction (arrow) on the surface of normal pyramidal cells.(b) The paracetamol treated group showing faint weak SYN reaction (arrow) on the surface of affected pyramidal cells.(c) The paracetamol+ silymarin treated group showing a moderate SYN reaction (arrow) on the surface of normal pyramidal cells. (SYN immunostain, x400, scale bar = 20 µm).

Three month-old age groups (adult groups)

A) Light microscopic study

Examination of the Cresyl violet-stained sections in the control group displayed the normal histological structure of the different layers of mPFC (**Fig.9 a**). The pyramidal and granular cells had a deeply stained Nissl substance (**Fig.9 b**).

The paracetamol treated group showed an apparently hypocellularity in different layers of mPFC(**Fig. 9c**). Lightly stained Nissl granules appeared in the pyramidal cells and granular cells. Also, some densely shrunken pyramidal cells, cellular infiltration and poorly stained neuropil were observed (**Fig. 9d**).

The paracetamol+ silymarin treated group revealed a partial restoration of the normal appearance of the layers of mPFC (Fig.9 e). The pyramidal and granular cells had moderately stained Nissl granules with a normal stained neuropil. Some dense shrunken pyramidal cells, dilated blood vessels and microglial cells with a dilated perineuronal space were also found (**Fig. 9f**).

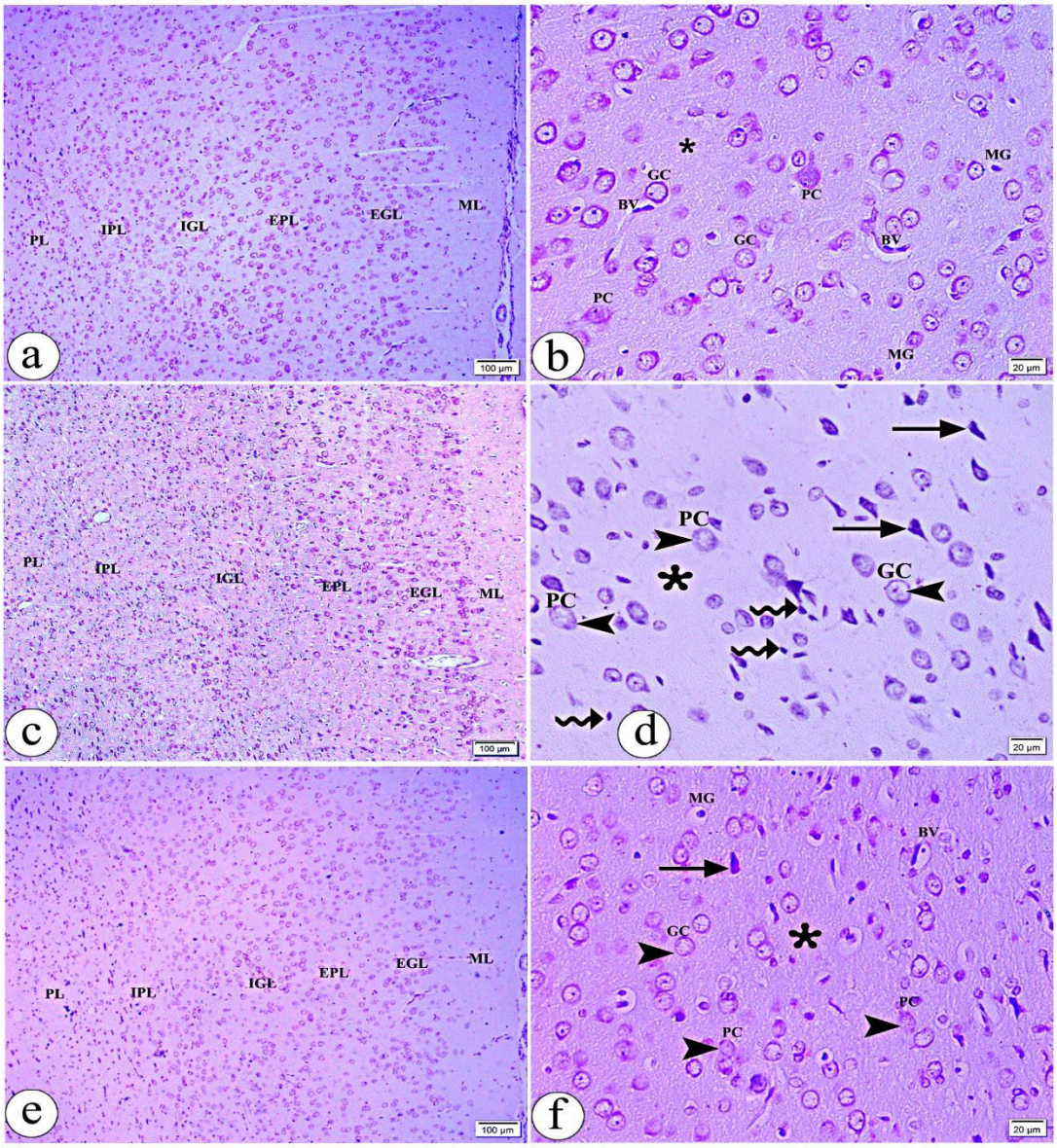

Fig. 9. The photomicrographs of the sections in the medial prefrontal cortex of three month-old rats in (a) The control group showing the layers of mPFC: the molecular layer (ML), the external

granular layer (EGL), the external pyramidal layer (EPL), the internal granular layer (IGL), the internal pyramidal layer (IPL) and the polymorphic layer (PL). (b) The control group showing deeply stained Nissl in the pyramidal cells (PC) and granular cells (GC). Notice normal blood vessel (BV), intact neuropil (asterisk) and microglial cells (MG). (c) The paracetamol treated group showing apparent hypocellularity in different layers of mPFC. (d) The paracetamol treated group showing lightly stained Nissl granules (arrowhead) in the pyramidal cells (PC) and granular cells (GC). Also, some dense shrunken pyramidal cells (arrow), cellular infiltration (curved arrow) and poorly stained neuropil (asterisk) are observed. (e)The paracetamol+ silymarin treated group showing restoration of the normal appearance of the layers of mPFC. (f) Paracetamol+ silymarin treated group showing that the pyramidal cells (PC) and granular cells (GC) have moderately stained Nissl granules (arrowhead). Some dense shrunken pyramidal cells (arrow) are seen. Notice dilated blood vessels (BV), microglial cells (MG) with dilated perineuronal space and normally stained neuropil (asterisk). (Cresyl fast violet stain, a, c, e X100, scale bar = 100 µm & b, d, f x400, scale bar = 20 µm).

The toluidine blue-stained sections demonstrated that the control group had intact pyramidal cells, intact processes, normal blood vessel within the intact neuropil (**Fig. 10a**).

The paracetamol treated group showed deformed pyramidal cells with irregularly shaped cell bodies. Some pyramidal cells appeared shrunken and deeply stained with an irregular outline and surrounded by haloes. Moreover, many vacuolations appear in the neuropil (**Fig. 10b**).

Paracetamol+ silymarin treated group displayed that the pyramidal cells appeared normal with vesicular nuclei, pale cytoplasm and intact apical dendrites. However, some pyramidal cells appeared shrunken and deeply stained with irregular outline and surrounded by haloes (**Fig. 10c**).

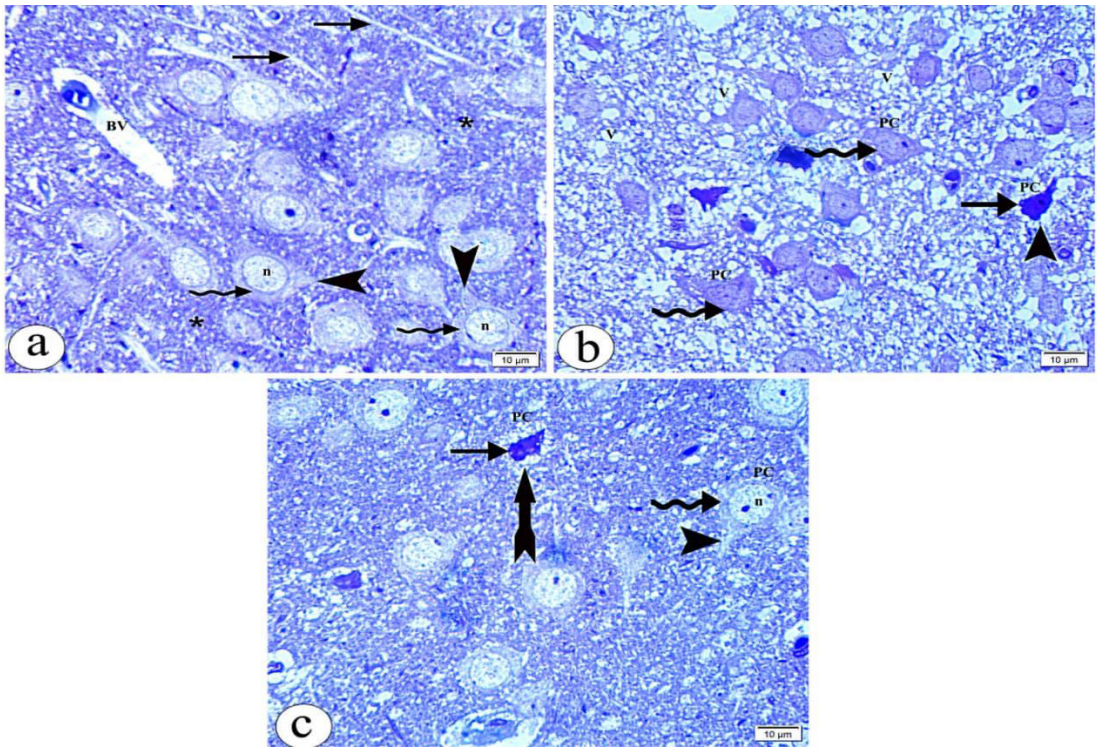

Fig. 10. The photomicrographs of the semithin sections in the medial prefrontal cortex of three month-old rats in (a) The control group showing intact pyramidal cells have vesicular nuclei (n) framed by pale cytoplasm (curved arrow) that having basophilic Nissl's granules with intact apical dendrites (arrowhead). Some intact processes (arrow), normal blood vessel (BV) within the intact neuropil (asterisk) can be observed. (b) The paracetamol treated group showing deformed pyramidal

cells (PC) with irregular cell bodies (curved arrow). Some pyramidal cells (PC) appear shrunken and deeply stained (arrow) with an irregular outline and surrounded by haloes (arrow-head). Many vacuolations (V) appear in the neuropil. (c) The paracetamol+ silymarin treated group showing pyramidal cells (PC) which appear normal with vesicular nuclei (n), pale cytoplasm (curved arrow) and intact apical dendrites (arrowhead). Some pyramidal cells (PC) are shrunken and deeply stained (arrow) with an irregular outline and surrounded by haloes (tailed arrow). (Toluidine blue x1000, Scale bar= 10 µm).

B) Electron microscopic study:

Ultrathin sections in the control group revealed that the pyramidal cells had a normal histological structure (**Fig. 11a**). On the other hand, the pyramidal cells had a shrunken electron-dense nucleus with an electron dense cytoplasm, destructed swollen mitochondria and many vacuolations was detected in paracetamol treated group (**Fig. 11b**). Interestingly, the paracetamol+ silymarin treated group showed that the pyramidal cells had euchromatic nuclei with prominent nucleoli, normal-appearing mitochondria and free ribosomes. Also, perineural glial cells were noticed (**Fig. 11c**).

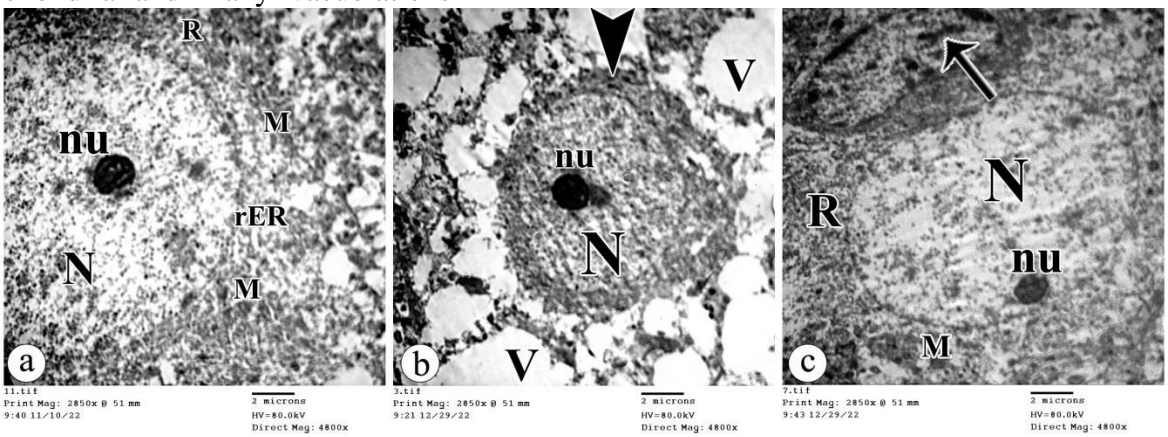

Fig. 11. The electron photomicrographs of the pyramidal cell of three month-old rats in (a) The control group showing a pyramidal cell having euchromatic nucleus (N) with a prominent nucleolus (nu). The cytoplasm shows mitochondria (M), rough endoplasmic reticulum (rER) and ribosomes (R). (b) The paracetamol treated group showing shrunken electron-dense nucleus (N), nucleolus (nu) with electron-dense cytoplasm (arrowhead), and many vacuolations (V). Also, loss of the cell organelles is observed. (c) The paracetamol+ silymarin treated group showing a normal pyramidal cell with euchromatic nucleus (N) and a prominent nucleolus (nu). The cytoplasm shows mitochondria (M) and ribosomes (R). The perineural glial cell was also viewed (arrow). (TEM x4800, Scale bar= 2 µm)

C) **Immunohistochemical study**:

Immunohistochemical evaluation demonstrated that the control group had a strong SYN reaction on the surface of normal pyramidal cells (**Fig.12a**). On the contrary, a weak SYN reaction on the surface of affected pyramidal cells was detected in the paracetamol treated group (**Fig. 12b**). The paracetamol+ silymarin treated group showed a moderate SYN reaction on the surface of normal pyramidal cells (**Fig. 12c**).

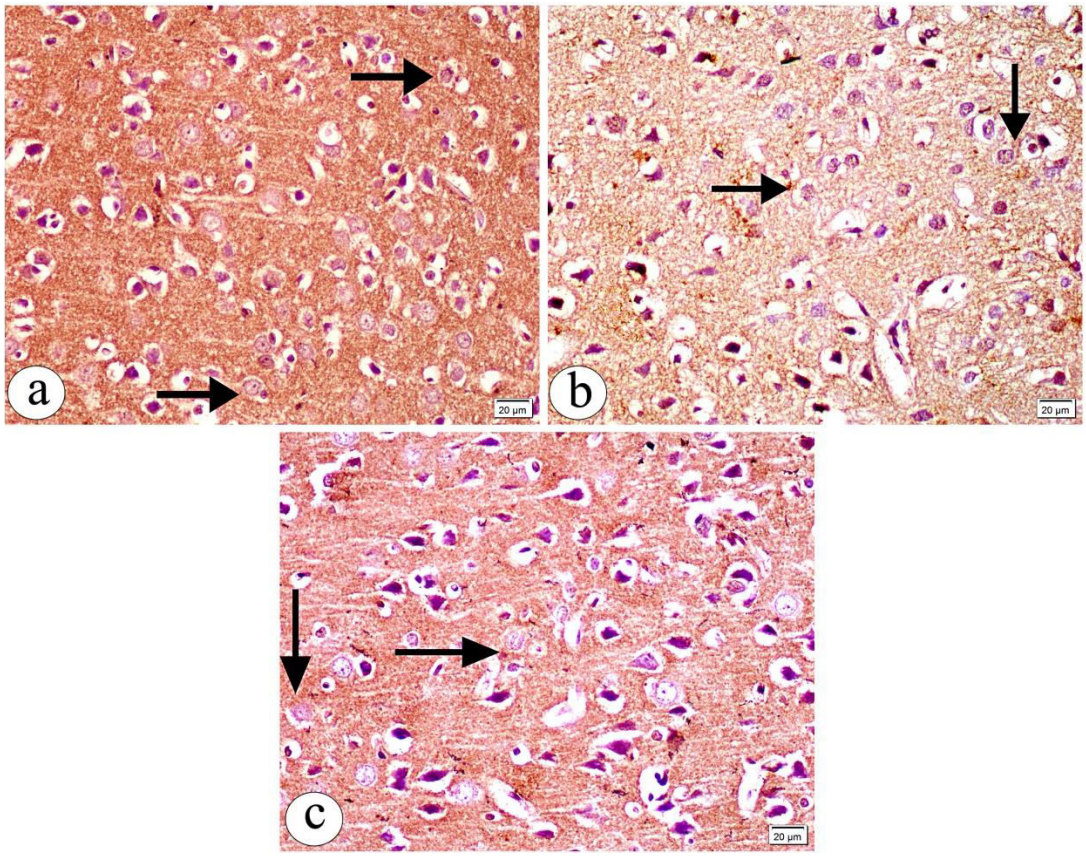

Fig. 12. The photomicrographs of the sections in the medial prefrontal cortex of three month-old rats in (a) The control group showing a strong SYN reaction (arrow) on the surface of normal pyramidal cells. (b) The Paracetamol treated group showing a weak SYN reaction (arrow) on the surface of affected pyramidal cells. (c) The Paracetamol+ silymarin treated group showing a moderate SYN reaction (arrow) on the surface of normal pyramidal cells. (SYN immunostaining, x400, scale bar = 20 µm)

Morphometric results

Statistical analysis demonstrated that the number of the pyramidal cells, the thickness of the mPFC and the area percent of SYN immunoexpression were significantly decreased in the paracetamol treated rats in different age groups when compared to the control rats (**Table 1**). On the other hand, all previously mentioned parameters were significantly increased in the paracetamol+ silymarin treated rats in different age groups when compared to the paracetamol treated rats (**Table 1**).

Discussion

One of the most often used medications in the world is paracetamol, an over-the-counter analgesic and antipyretic **(Brune et al., 2015).** It recommended for the treatment of fever and pain in the pregnant women and children. Moreover, it is easily accessible for newborns in an over-the-counter form that is marketed as "safe, mild, and effective" and comes with no warnings about side effects other than allergic responses **(Chambers, 2015)**.

Numerous researches have been conducted on the harmful effects of paracetamol on the liver **(Hota et al., 2022 and Igeh et al.,2022)**, kidney **(Akpan et al., 2022 and Shams &**

Elmesiry, 2022), and testis (**Blecharz-Klin et al., 2022**). Previous study reported that exposure to paracetamol during pregnancy induced olfactory loss and decreased emotionality in rat pups which are related to incidence of neurodevelopmental disorders like autism and ADHD (**Rigobello et al., 2021).** In addition, many studies were concluded that silymarin has preventive properties against paracetamol-induced hepatotoxicity (**Girish et al., 2009 and Ahmad et al., 2019**).

Table 1. The number of pyramidal cells, the mPFC thickness and the area percent (%) of SYN immunoreaction in all studied groups

Parameters / Groups	Number of pyramidal cells	The mPFC thickness (µm)	Area % of SYN immunoreaction
Subgroup IA	66.40± 3.05	292.2± 10.01	18.00±0.4942
Subgroup IB	64.05± 3.42	293.7± 10.55	17.59±0.86
Subgroup IC	39.70 ±2.29[a, b]	275.2± 5.01 [a, b]	8.09±0.67 [a, b]
Subgroup ID	65.40±4.98[c]	304.5±9.85 [a, b,c]	15.96±1.26 [a, b,c]
P-value	< 0.0001*	< 0.0001*	< 0.0001*
Subgroup IIA	89.30±6.17	653.2±10.48	32.95±1.77
Subgroup IIB	86.95±6.03	650.4±10.61	32.60±1.94
Subgroup IIC	45.70±2.43 [a, b]	578.1±12.46 [a, b]	21.66±2.63 [a, b]
Subgroup IID	72.70±4.70 [a, b,c]	682.9±13.89 [a, b,c]	26.71±1.64 [a, b,c]
P-value	< 0.0001*	< 0.0001*	< 0.0001*
Subgroup IIIA	59.70±4.23	1053±72.36	37.88±3.54
Subgroup IIIB	59.65±3.79	1051.2±67.03	37.13±3.65
Subgroup IIIC	40.00±2.75 [a, b]	933.6±68.00 [a, b]	12.82±1.36 [a, b]
Subgroup IIID	46.90±2.45 [a, b,c]	961.2±70.08 [a, b]	41.35±2.31 [a, b,c]
P-value	< 0.0001*	< 0.0001*	< 0.0001*

Data are represented as Mean ± SD.* means statistically significant difference.
[a] statistically significant as compared with the subgroup A , P < 0.05
[b] statistically significant as compared with the subgroup B, P < 0.05
[c] statistically significant as compared with the subgroup C, P < 0.05

However, there are few reports on how paracetamol affects the brain and up to date no study on its effect on mPFC. The aim of the current study was to clarify how early prenatal and postnatal exposure to paracetamol affected the rat's medial prefrontal cortex histologically and potential role of silymarin.

In order to avoid hormonal effects in females such as estrogen strengthening cellular proliferation throughout the cycle and leading to an increase in immature neurons in the cortex, only male albino rats were used in the current study (**El-Safti et al., 2017).**

The present findings revealed that the fetal paracetamol exposure causes an abnormal morphology of mPFC, apparently hypocellularity in different layers, interference with the cortical development, degenerated and vacuolated pyramidal cells. These findings were confirmed statistically by significant decrease in the numbers of the pyramidal cells and thickness of mPFC. The present findings were

supported a previous study concluded that some infants and children are at risk for paracetamol-induced neurological impairment, and postnatal paracetamol exposure in those vulnerable infants and children is the cause of many, if not most, occurrences of ASD **(Patel et al., 2022).**

The proposed mechanisms by which paracetamol could interrupt the normal development of mPFC are endocrine disruption, endocannabinoid receptor activation during development **(Schultz, 2010)**, and inflammation **(Parker et al., 2017)**.Moreover, paracetamol interacted with the cyclooxygenase system, which may have caused developmental neurotoxicity **(Blaise et al., 2018).** Additionally, early paracetamol treatment caused oxidative stress indicators to be more abundant and the transcription of genes encoding a receptor involved in neurogenesis to be reduced **(Philippot et al., 2017).**

The hypothesis that paracetamol is specifically harmful to neurons is a more straightforward one. Posadas et al. investigated this by administering paracetamol to rat cortical neurons in vivo, evaluating the amount of resulted neuronal loss and found that paracetamol was moderately toxic to the cortical neurons **(Posadas et al., 2010).**

Moreover, former researchers concluded that the developmental neurotoxicity of paracetamol is enough to influence rats' adult learning, memory, cognition, and spontaneous behavior if the exposure to this drug occurs throughout the brain growth spurt's peak. Also, it was provided a validation to the rodent model that is applicable to human **(Philippot et al., 2018 and Hussin & Al-Allaf, 2022).**

In the same context, it was found that prenatal and early paracetamol exposure caused significant alterations in in dopaminergic and serotonergic neurotransmission in the prefrontal cortex as well as decline in the cognitive functions concerning spatial memory processing. In addition, the level of cerebral taurine was dramatically lowered after paracetamol treatment **(Blecharz-Klin et al., 2017)**. Emerging evidence suggests that taurine indirectly modulated neuronal activity and the processing of information in the frontal cortex and hippocampus formation because taurine acts as an endogenous agonist at the glycine receptors **(Mori et al., 2002)**.

Treatment with paracetamol also revealed elevated aspartic acid levels in the prefrontal cortex which has an established role in the development of the central nervous system and participation in the synaptic plasticity, memory, and spatial learning processes **(Topo et al., 2010).** This might be linked to decrease in SYN reaction observed in this study. Finally, it has been established that paracetamol suppresses the activity of Na+K+ ATPase and Mg2+ ATPase in the fetal human brain, which may have an impact on the release and uptake of biogenic amines and the CNS's development **(Sarkar et al., 1989).**

Nissl staining using cresyl fast violet is considered as an easy and rapid screen for neurodegeneration and the morphology of the dying neurons may be an indication of occurrence of apoptosis **(Muonagolu & Ekong, 2016).** Nissl bodies are granular substances presents in neurons, and their function is to produce and release proteins for intra- and inter-cellular usage. Under different physiological and pathological circumstances, Nissl substance exhibits changes and may dissolve or vanish **(Ajayi et al., 2022).**

Loss of Nissl bodies after

paracetamol administration in 21-days aged and adult groups was observed in this work, so we have suggested that paracetamol administration induced chromatolysis. Chromatolysis is the breakdown of the Nissl bodies within a neuron's cell body. Chromatolysis may be brought on by cell toxicity caused by the generation of free radicals by external substances, axotomy, ischemia, or exhaustion as well as viral infections that cause Nissl substances to disintegrate. Chromatolysis can follow by a neuronal regeneration or usually precedes an apoptosis which will eventually cause a structural and functional loss **(Akinola et al., 2011)**. In addition, loss of Nissl bodies diminished the pyramidal neurons' activity and rough endoplasmic reticulum's synthesis which in turn may indicate that these animals' cognitive function has been compromised **(Akinola et al., 2011).**

Our findings were in agreement with **Hussin, and Al-Allaf, 2022** who reported that paracetamol administration at a dose of 60 mg/kg /day to male rat pups from postnatal day 7 till postnatal day 14 led to irregularity in the shape of the pyramidal cells with deeply stained nuclei, karyolysis and dilated blood vessel in the frontal cortex **(Albo-Hussin & Al-Allaf, 2022).**

On the other hand, previous authors found that treatment with 100, 200, 400 mg/kg paracetamol for 11 days displayed no histological change in the structure of the prefrontal cortex in adult male Sprague Dawley rats. Additionally, in behavioral activities, there were no differences in anxiety levels **(Karakilic et al., 2022).**

Observed dilated congested blood vessels and inflammatory cellular infiltration in paracetamol treated rats might be attributable to the inflammatory process followed the cellular degeneration **(Jeon , 2014).** The appearance of wide perineuronal space in this study was mostly due to the dissolution of the cytoskeletal components of the pyramidal cells, which causes neurons to shrink and withdraw their cytoplasmic processes **(Abd Elaziz & Laag, 2018).**

Our ultrastructural examination demonstrated the cytoarchitectural affection of pyramidal cells in the paracetamol treated rats in 21-days aged and adult rats showed heterochromatic shrunken nuclei, loss of the cell organelles and many vacuolations. Also, destructed swollen mitochondria were detected in many pyramidal cells.

Observed nuclear damage may suggest that paracetamol treatment led to degeneration of DNA, thereby followed by apoptosis. Because DNA is necessary for transcription and replication, nuclear degradation will affect the function of the neurons of the mPFC **(Etibor, 2015). Kalinichenko, et al.** reported that late phase of apoptosis result in the cytoplasm losing its structure and the nucleus showing greater degeneration **(Kalinichenko et al., 2023)**. Cell shrinkage, chromatin clumping, membrane budding, nuclear pyknosis (shrinkage), and karyorrhexis (nuclear fragmentation) are characteristic morphological alterations of apoptosis. When a cell receives a death signal from the outside or inside, apoptosis begins. These signals trigger the cell surface death receptor and mitochondrial pathways, which are two of the main apoptotic mechanisms. Phagocytes remove the apoptotic bodies from the tissue that are formed when apoptotic cells divide **(Zararsiz, 2006).**

The present alterations in the mitochondria exhibited the neurons' response to the free radicals generated

from tissue injury **(Eru et al., 2022).** Additionally, mitochondria are crucial to the apoptotic process. Apoptosis is brought on by death signals that increasing the outer mitochondrial membrane's permeability **(Ashafaq et al., 2022).**

Accumulating evidence in this study suggests that PFC damage adversely affects recognition memory tasks. According to general definitions, recognition memory is the capacity to determine whether a thing or an event has already happened **(Alghamdi, 2022).**

The immunoactivity of synaptophysin protein was evaluated immunohistochemically to determine the amount of this protein, which is essential for the synaptic plasticity in the neurons of the mPFC. It was demonstrated that SYN expression was significantly decreased in the mPFC of all animals treated with paracetamol. Therefore, it is reasonable to suggest that the paracetamol treatment disrupt synapse development, density, and integration in the mPFC.

Synaptophysin is an essential membrane glycoprotein found in the presynaptic vesicles of all neurons in the brain. It contributes to the development of synapses, synaptic biogenesis, starting the release of neurotransmitters, and transmission of the synaptic vesicle endocytosis **(Zhou et al., 2023).** In the absence of this protein, a nerve cell can only send a nerve impulse many times before running out of the synaptic vesicles. The recurrent conduction of a nerve impulse is the foundation of cognitive processes; hence mutations in the synaptophysin gene are frequently seen in the mental retardation. Moreover, decreased expression of SYN indicates axonal damage **(Zimatkin, 2017).**

The present morphometric analysis demonstrated that in all test groups, the number of the pyramidal cells in the mPFC and the cortical thickness in the paracetamol treated animals decreased significantly when compared to the control group. These results are supported by another study on the rats which received 60 mg/kg /day of paracetamol from PND 7 to PND 14, which concluded that paracetamol induced destruction of neurons resulted in a glaring decrease in the thickness of the pyramidal layer of Cornue Ammonis **(Hussin & Al-Allaf, 2022).**

In different age groups, silymarin induced a remarkable improvement in the architecture of mPFC and ultrastructure of the pyramidal cells with concurrent increase in the number of the pyramidal cells and the thickness of the mPFC on histomorphometrical analysis. The protective ability of silymarin was evident from partially restored Nissl granules staining, relieved blood vessel congestion, reduced nuclear and cytoplasmic degeneration as well as increased SYN immunoexpression. These findings suggested that silymarin treatment had beneficial impacts on the paracetamol toxicity in the mPFC.

The ultrastructural improvement of the pyramidal cells induced by silymarin in this study may be linked to its ability to penetrate the nucleus and boost ribosomal protein synthesis by activating RNA polymerase I and rRNA transcription in the nucleus as well as increasing the synthesis of ribosomes **(Adelina, 2022).** Furthermore, silymarin increases the strength of plasma membranes as well as prevents membranes from rupturing because it is lipophilic and strongly binds to plasma membrane components **(Basiglio, 2009).**

Our finding was supported by Onaolapo et al. who found that

paracetamol treatment was associated with structural evidence of cerebral cortex injury, reduced weight gain, working-memory impairment, and anxiety and pre-administration of silymarin partially reduced the effects of paracetamol toxicity **(Onaolapo et al., 2017)**. Also, this is in the same line with the previous research that concluded that hepatic encephalopathy, which damages the liver and the brain, is prevented by silymarin at a dose of 100 mg/kg/Day **(Teksoy et al., 2020).**

According to studies, silymarin has significant neuroprotective properties against several neurodegenerative conditions, including Alzheimer's and Parkinson's diseases. This is largely because of its ability to reduce oxidative stress in the brain, reduce inflammation, inhibit apoptosis **(Scorticati et al., 2004),** and the potential to enhance learning, memory and cognitive function **(Spencer, 2009).**

Silymarin is considered as a potent antioxidant molecule because it has methoxy moiety connected to the phenolic rings in its polyphenolic structure. Moreover, according to some reports, silymarin has a greater capacity for neutralizing free radicals than vitamin E **(Surai, 2015).** Additionally, it mitigates lower levels of dopamine and serotonin in the prefrontal cortex **(Pérez-H, 2014)**. Moreover, by raising the activity of enzymatic and nonenzymatic antioxidant markers, the reduction of free radicals, secretase, butyrylcholinesterase, and cholinesterase silymarin can counter paracetamol-induced neurotoxicity in the animal models **(Borah, 2013)**. Furthermore, it stimulates neurogenesis, improves the vascular blood flow in frontal cortex and striatum **(Raza et al., 2011)** and suppressed microglial activation **(Tabaa, et al., 2022).**

In the prefrontal cortex, treatment with silymarin in depression-induced model can restore cortical lipid peroxidation and significantly reduced the levels of pro-inflammatory cytokines impeding neuroinflammation. Pro-inflammatory cytokines can damage neural plasticity, which is linked to decreased neurogenesis and altered signaling of neurotrophic factors which reinforcing neuronal survival and differentiation **(Song et al., 2016; Ashraf, 2019 and Bahram et al., 2020).**

Conclusion

Collectively, the evidence obtained from this study indicated that paracetamol treatment at a dose of 350 mg/kg /day during pregnancy and lactation caused neuronal damage in the mPFC' s cellular profile of the developing and adult nervous system. Silymarin appeared to have been beneficial in protecting and preserving the mPFC' structure and reducing the deleterious effect of paracetamol. Therefore, we have suggested that paracetamol treatment might induce impairments in learning and behavior. Even though paracetamol is frequently used during pregnancy and the first few years of life due to its advantages over other analgesics, a balanced risk assessment based on the best expert judgment is required and should be given priority. Future studies on paracetamol should focus to demonstrate its biochemical and molecular mechanisms that led to the mPFC histological disruption.

References

- **Abd Elaziz H O, Laag E M (2018)**. Histological study of the possible protective action of omega-3-fatty acids on the injurious effect induced by

Bisphenol A on rat hippocampus. Egyptian Journal of Histology, 41(1): 39-54.

- **Adedayo A D, Stephen A O, Adekilekun T A, Daniel A T (2017)**. Lead induces inflammation and neurodegenerative changes in the rat medial prefrontal cortex. Anatomy, 11(2): 79-86.
- **Adelina J A M (2022).** Clinical Studies of Silymarin as a Protective Agent Against Liver Damage Caused by Anti-TB Drugs, Methotrexate, and in Cases of Chronic Hepatitis C and Diabetes Mellitus. Pharmacognosy Journal, 14(2): 358-368.
- **Ahmad M M, Rezk N A, Fawzy A, Sabry M (2019).** Protective effects of curcumin and silymarin against paracetamol induced hepatotoxicity in adult male albino rats. Gene, 712: 143966.
- **Ajayi A M, Melete J, Ben-Azu B, Umukoro S (2022).** Aggressive-like behaviour and neurocognitive impairment in alcohol herbal mixture-fed mice are associated with increased neuroinflammation and neuronal apoptosis in the prefrontal cortex. Journal of biochemical and molecular toxicology, e23252.
- **Akinola O B, Omotoso O G, Dosumu O O, Akinola O S, Olotufore F (2011).** Diabetes-induced prefrontal Nissl substance deficit and the effects of neem-bitter leaf extract treatment. International Journal of Morphology, 29(3): 850-856.
- **Akpan D F, Umoh A R, Tologbonse A A, Akpan J S O, Offor J S (2022).** Ameliorating Effects of Solanum anomalum Thonn. Ex. Schumach Fruit Extracts on Paracetamol-induced Kidney Injuries and Haemato-biochemical Indices in Rats. Journal of Complementary and Alternative Medical Research, 17(1): 37-45.
- **Albo-Hussin S M Y, Al-Allaf L I K (2022)**. Histological evaluation of postnatal exposure to paracetamol impacts on cerebral cortex histology of male rat. In AIP Conference Proceedings ,2547 (1): 020024. AIP Publishing LLC.
- **Alemany S, Avella-García C, Liew Z, García-Esteban R, Inoue K, Cadman T,et al. (2021).** Prenatal and postnatal exposure to acetaminophen in relation to autism spectrum and attention-deficit and hyperactivity symptoms in childhood: Meta-analysis in six European population-based cohorts. European Journal of Epidemiology, 36: 993-1004.
- **Alghamdi B S, Alsufiani H M, Mansouri R A, Bahaidrah K A, Alzahrani N A (2022).** Investigation of the optimal dose for experimental lipopolysaccharide-induced recognition memory impairment: behavioral and histological studies. Journal of Integrative Neuroscience, 21(2): 49.
- **Ashafaq M, Hussain S, Alshahrani S, Madkhali O, Siddiqui R, Khuwaja G, et al. (2022).** Role of cinnamon oil against acetaminophen overdose induced neurological aberrations through brain stress and cytokine upregulation in rat brain. Drug and chemical toxicology, 45(2): 633-640.
- **Ashraf A, Mahmoud P A, Reda H, Mansour S, Helal M H, Michel H E, et al. (2019).** Silymarin and silymarin nanoparticles guard against chronic unpredictable mild stress induced depressive-like behavior in mice: involvement of neurogenesis and

NLRP3 inflammasome. . Journal of Psychopharmacology, 33(5): 615-631.

- **Bahram Kalhori P, Hajizade Moghaddam A, Zare M, Sayrafi R (2020).** The effect of silymarin on memory and learning disorders induced by TiO2 nanoparticles. Daneshvar Medicine, 25(5): 39-44.
- **Basiglio C L, Pozzi E S, Mottino A D, Roma M G (2009).** Differential effects of silymarin and its active component silibinin on plasma membrane stability and hepatocellular lysis. . Chemico-Biological Interactions, 179(2): 297–303.
- **Beier K T, Steinberg E E, DeLoach K E, Xie S, Miyamichi K, Schwarz L, et al (2015).** Circuit architecture of VTA dopamine neurons revealed by systematic input-output mapping. Cell, 162(3): 622-634.
- **Blaise J H, Park J E, Bellas N J, Gitchell T M, Phan V (2018).** Caffeine consumption disrupts hippocampal long-term potentiation in freely behaving rats. Physiological reports, 6(5): e13632.
- **Blecharz-Klin K, Piechal A, Jawna-Zboińska K, Pyrzanowska J, Wawer A, Joniec-Maciejak I, et al. (2017)**. Paracetamol− Effect of early exposure on neurotransmission, spatial memory and motor performance in rats. Behavioural Brain Research, 323: 162-171.
- **Blecharz-Klin K, Sznejder-Pachołek A, Wawer A, Pyrzanowska J, Piechal A, Joniec-Maciejak I, et al. (2022).** Early exposure to paracetamol reduces level of testicular testosterone and changes gonadal expression of genes relevant for steroidogenesis in rats offspring. Drug and chemical toxicology, 45(4): 1862-1869.
- **Borah A, Paul R, Choudhury S, Choudhury A, Bhuyan B, Das Talukdar A, et al. (2013).** Neuroprotective potential of silymarin against CNS disorders: insight into the pathways and molecular mechanisms of action. . CNS neuroscience & therapeutics, 19(11): 847-853.
- **Brune K, Renner B, Tiegs G (2015).** Acetaminophen/paracetamol: a history of errors, failures and false decisions. European Journal of Pain, 19(7): 953-965.
- **Chambers C (2015).** Over-the-counter medications: risk and safety in pregnancy. Seminars in perinatology, 39(7), 541–544.
- **El-Din M S, Eid M, Talaat W (2014).** Micellar liquid chromatographic determination of ribavirin, silybin, interferon α 2A, lamivudine, and ursodeoxycholic acid in dosage forms and biological fluids. Journal of Liquid Chromatography & Related Technologies, 37(13): 1785-1804.
- **El-Safti F E N A, El-Kholoy W B, El-Mehi A E, Selima R R (2017).** A comparative study on the effect of aging on the hippocampal CA1 area of male albino rat. Menoufia Medical Journal, 30(4): 1079.
- **Eru E M, Anani S E, Paulinus S O, Mesembe O E, Uruakpa K C, Umoh N M,et al. (2022).** Scopolamine-Induced Alzheimer's Disease in Wistar Rats: Aqueous Talinum Triangulare Potency on the Hippocampal Nissl Bodies and Long-Term Learning and Memory. Tropical Journal of Natural Product Research, 6(1): 117-122.
- **Essiet G, Essien A, Akuodor G J I (2017).** Embryotoxic and

teratogenic potentials of Salacia lehmbachii in rats. 12: 43-48.

- **Etibor T A, Ajibola M I, Buhari M O, Safiriyu A A, Akinola O B, Caxton-Martins E A (2015).** Datura metel Administration Distorts Medial Prefrontal Cortex Histology of Wistar Rats. World Journal of Neuroscience, 5: 282-291.
- **Girish C, Koner B C, Jayanthi S, Ramachandra Rao K, Rajesh B, Pradhan S C (2009).** Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. Fundamental & clinical pharmacology, 23(6): 735-745.
- **Hirayama K, Oshima H, Yamashita A, Sakatani K, Yoshino A, Katayama Y (2016).** Neuroprotective effects of silymarin on ischemia-induced delayed neuronal cell death in rat hippocampus. Brain research, 1646: 297-303.
- **Hota R, Nanda B, Behera B, Bose A (2022).** Ameliorative effect of ethanolic extract of Limnophila rugosa (Scrophulariaceae) in paracetamol-and carbon tetrachloride-induced hepatotoxicity in rats. Future Journal of Pharmaceutical Sciences, 8(1): 6.
- **Hussin S A, Al-Allaf L (2022).** Histological changes of CA and DG regions of hippocampus of rats' brain after exposure to Acetaminophen in postnatal period. Iraqi Journal of Veterinary Sciences, 36(1): 151-158.
- **Igeh P C, Ishaku E, Nangbes J G, Choji S, Okonkwo F O (2022).** Aqueous Extract of Erythrina senegalensis Exhibits Dose-Dependent Hepatoprotective Activity on Paracetamol-Induced Liver Damage in Wistar Rats. Advances in Biological Chemistry, 12(2): 48-60.
- **Jeon HJ, S M, Choi HS, Lee OH, Lee BY (2014).** Gelidium elegans, an edible red seaweed, and hesperidin inhibit lipid accumulation and production of reactive oxygen species and reactive nitrogen species in 3T3-L1 and RAW264.7 cells. Phytother Res, 28(11): 1701–1709.
- **Kalinichenko S G, Pushchin I I, Matveeva N Y (2023).** Neurotoxic and cytoprotective mechanisms in the ischemic neocortex. Journal of Chemical Neuroanatomy, 102230.
- **Karakilic A, Kizildag S, Yuce Z, Celik Y S, Kandis S, Bozan H R, et al. (2022).** Repeated acetaminophen administration damaged hippocampal tissue but did not affect prefrontal cortex or anxiety behaviors. Acta Neurobiol Exp, 82: 170-178.
- **Khazaei R, Seidavi A, Bouyeh M (2022).** A review on the mechanisms of the effect of silymarin in milk thistle (Silybum marianum) on some laboratory animals. Veterinary Medicine and Science, 8(1): 289-301.
- **Kuo J (2007).** Electron microscopy: methods and protocols (Vol. 369): Springer Science & Business Media.
- **Lu P, Mamiya T, Lu L, Mouri A, Zou L, Nagai T,et al. (2009).** Silibinin prevents amyloid β peptide-induced memory impairment and oxidative stress in mice. British journal of pharmacology, 157(7): 1270-1277.
- **Mahmoud F Y (2001).** Histological effects of gentamicin intake during pregnancy on the liver and kidney of the fetus and

mother of albino rat. The Egyptian Journal of Anatomy, 24(1): 35-57.

- **Miguel P M, Deniz B F, Deckmann I, Confortim H D, Diaz R, Laureano D P, et al. (2018).** Prefrontal cortex dysfunction in hypoxic-ischaemic encephalopathy contributes to executive function impairments in rats: potential contribution for attention-deficit/hyperactivity disorder. The World Journal of Biological Psychiatry, 19(7): 547-560.
- **Mori M, Gähwiler B H, Gerber U (2002).** β-Alanine and taurine as endogenous agonists at glycine receptors in rat hippocampus in vitro. The Journal of physiology, 539(1): 191-200.
- **Muonagolu N J, Ekong M B (2016).** Allium sativum alters the cyto-architecture of the medial prefrontal cortex and neurobehaviour of adult Wistar rats. Nigerian Journal of Neuroscience Nig. J. Neurosci, 7(2): 53-58.
- **Onaolapo O J, Adekola M A, Azeez T O, Salami K, Onaolapo A Y (2017).** l-Methionine and silymarin: A comparison of prophylactic protective capabilities in acetaminophen-induced injuries of the liver, kidney and cerebral cortex. Biomedicine & Pharmacotherapy, 85: 323-333.
- **Parasuraman S, Raveendran R, Mueen Ahmed K (2015).** Violation of publication ethics in manuscripts: Analysis and perspectives. Journal of Pharmacology and Pharmacotherapeutics, 6(2): 94-97.
- **Parker W, Hornik C. D, Bilbo S, Holzknecht Z E, Gentry L, Rao R, et al. (2017).** The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. Journal of International Medical Research, 45(2): 407-438.
- **Patel E JP, Bono-Lunn D, Kuchibhatla M, Palkar A, Sarafian J, et al. (2022).** The safety of pediatric use of paracetamol (acetaminophen): a narrative review of direct and indirect evidence. Minerva Pediatrics, 74(6), 774–788.
- **Pérez-H J, Carlos Carrillo-S Esperanza G, Gabriela R M, Pérez-Tamayo R, Anahí C (2014)**. Neuroprotective effect of silymarin in a MPTP mouse model of Parkinson's disease. Toxicology, 319: 38-43.
- **Peters A J, Marica A M, Fabre J M, Harris K D, Carandini M (2022).** Visuomotor learning promotes visually evoked activity in the medial prefrontal cortex. Cell Reports, 41(3): 111487.
- **Philippot G, Gordh T, Fredriksson A, Viberg H (2017)**. Adult neurobehavioral alterations in male and female mice following developmental exposure to paracetamol (acetaminophen): characterization of a critical period. Journal of Applied Toxicology, 37(10): 1174-1181.
- **Philippot G, Hallgren S, Gordh T, Fredriksson A, Fredriksson R, Viberg H (2018)**. A cannabinoid receptor type 1 (CB1R) agonist enhances the developmental neurotoxicity of acetaminophen (paracetamol). Toxicological Sciences, 166(1): 203-212.
- **Posadas I, Santos P, Blanco A., Munoz-Fernandez M, Cena V (2010).** Acetaminophen induces apoptosis in rat cortical neurons. PLoS ONE, 5(12):e15360.
- **Rao P R, Viswanath R K (2007).** Cardioprotective activity of silymarin in ischemia-reperfusion-

induced myocardial infarction in albino rats. Experimental & Clinical Cardiology, 12(4): 179.

- **Raza S S, Khan M M, Ashafaq M, Ahmad A, Khuwaja G, Khan A, et al. (2011).** Silymarin protects neurons from oxidative stress associated damages in focal cerebral ischemia: a behavioral, biochemical and immunohistological study in Wistar rats. Journal of the Neurological Sciences, 309(1-2): 45-54.
- **Rigobello C, Klein R M, Debiasi J D, Ursini L G, Michelin A P, Matsumoto A K, et al. (2021).** Perinatal exposure to paracetamol: Dose and sex-dependent effects in behaviour and brain's oxidative stress markers in progeny. Behavioural Brain Research, 408: 113294.
- **Sarkar A, Chakraborti A, Saha U, Bose S, Sengupta D (1989)**. Effects of aspirin and paracetamol on ATPases of human fetal brain: an in vitro study. Indian Journal of Experimental Biology, 27(9): 802-804.
- **Sauer J F, Folschweiller S, Bartos M (2022).** Topographically organized representation of space and context in the medial prefrontal cortex. Proceedings of the National Academy of Sciences, 119(6):e2117300119.
- **Schultz S T (2010).** Can autism be triggered by acetaminophen activation of the endocannabinoid system? Acta neurobiologiae experimentalis, 70(2): 227-231.
- **Scorticati C, Prestifilippo J P, Eizayaga F X, Castro J L, Romay S, Fernández M A, et al. (2004).** Hyperammonemia, brain edema and blood-brain barrier alterations in prehepatic portal hypertensive rats and paracetamol intoxication. World journal of gastroenterology, 10(9):1321.
- **Senturk H, Kabay S, Bayramoglu G, Ozden H, Yaylak F, Yucel M,et al. (2008)**. Silymarin attenuates the renal ischemia/reperfusion injury-induced morphological changes in the rat kidney. World Journal of Urology, 26: 401-407.
- **Shams G, Elmesiry S (2022).** The Possible Protective Effect of Galantamine against Paracetamol Induced Hepatic and Renal Toxicity in Rats. Zagazig Veterinary Journal, 50(3): 219-227.
- **Song P, Zha M, Yang Q, Zhang Y, Li X, Rudan I (2021)**. The prevalence of adult attention-deficit hyperactivity disorder: A global systematic review and meta-analysis. Journal of global health, 11: 22-28.
- **Song X, Zhou B, Zhang P, Lei D, Wang Y, Yao G,et al. (2016).** Protective effect of silibinin on learning and memory impairment in LPS-treated rats via ROS–BDNF–TrkB pathway. Neurochemical research, 41: 1662-1672.
- **Spencer J P (2009)**. Flavonoids and brain health: multiple effects underpinned by common mechanisms. Genes & nutrition, 4(4): 243-250.
- **Surai P F (2015).** Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. Antioxidants, 4(1): 204-247.
- **Tabaa M M E, Aboalazm H M, Shaalan M, Khedr N F (2022)**. Silymarin constrains diacetyl-prompted oxidative stress and neuroinflammation in rats: involvements of Dyn/GDNF and MAPK signaling pathway.

Inflammopharmacology, 30(3), 961-980.

- **Teksoy O, Sahinturk V, Cengiz M, İnal B, Ayhancı A (2020).** The possible effects of silymarin on cerebrum with experimental hepatic encephalopathy in rats. Int J Res, 8: 140-146.
- **Topo E, Soricelli A, Di Maio A, D'Aniello E, Di Fiore M M, D'Aniello A (2010).** Evidence for the involvement of D-aspartic acid in learning and memory of rat. Amino acids, 38: 1561-1569.
- **Watanabe M (2016).** Emotional and motivational functions of the prefrontal cortex. Brain and Nerve= Shinkei Kenkyu no Shinpo, 68(11): 1291-1299.
- **Yang C, Hu Y, Talishinsky A D, Potter C T, Calva C B, Ramsey L A, et al. (2022).** Medial prefrontal cortex and anteromedial thalamus interaction regulates goal-directed behavior and dopaminergic neuron activity. Nature Communications, 13(1): 1386.
- **Yang J, Wang X Y, Xue J, Gu Z L, Xie M L (2013).** Protective effect of apigenin on mouse acute liver injury induced by acetaminophen is associated with increment of hepatic glutathione reductase activity. Food & function, 4(6): 939-943.
- **Zararsız İ, Kuş, İ, Çolakoğlu N, Pekmez H, Yılmaz H R, Sarsılmaz M (2004)**. Protective Effects of Melatonin Hormone Against Exposure of Formaldehyde-Induced Oxidative Damage in Lung of Rats: Light Microscopic and Biochemical Study. Van Medical Journal, 11(4): 105-112.
- **Zararsiz I, Kus I, Akpolat N, Songur A, Ogeturk M, Sarsilmaz M (2006).** Protective effects of ω-3 essential fatty acids against formaldehyde-induced neuronal damage in prefrontal cortex of rats. . Cell Biochemistry and Function, 24(3): 237–244.
- **Zhou Q, Lin L, Li H, Li Y, Liu N, Wang H, et al. (2023).** Intrahippocampal injection of IL-1β upregulates Siah1-mediated degradation of synaptophysin by activation of the ERK signaling in male rat. Journal of neuroscience research, 10.1002/jnr.25170. Advance online publication. https://doi.org/10.1002/jnr.25170
- **Zimatkin S M (2017).** Effects of Antenatal Alcoholization on Brain Cortex Neurons Postnatal Development in Rats. International Journal of Neuroscience and Behavior Studies,1(1): 7-17.