DOI:10.22059/IJVM.2024.377462.1005588 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554`

Neurotoxicity of Isotretinoin in Mice: Behavioral and Tissue Neurological Function Assessment

Running title: IsotretinoinNeurotoxicity in Mice

Y.Z. Alabdaly[1] , Sahar Khalid Abdul hameed[2], Nada Khalil Ibrahim[3]

1 Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

2 Al Nour University College, Mosul, Iraq.

3 Department of Anesthesiology, Medical Technical Institute, Northern Technical University, Mosul, Iraq.

Abstract

Background: Isotretinoin is used to treat some skin disorders in dogs and cats by reducing the size and activity of their sebaceous glands, although it may have some neurobehavioral side effects.

Objective: To evaluate isotretinoin's effects on the brain and neurotransmitters, as well as its impact on neurobehavior and motor activity.

Methods: A total of 15 mice were arranged into 3 groups: the 1st group was a control, the 2nd group received 125 mg/kg isotretinoin, and the 3rd group received 250 mg/kg orally.

Results: The LD50 for isotretinoin is 4841.2 mg/kg. The neurobehavioral measurements of mice reveal significant effects on changes in open field activity, time spent in dark areas, and negative geotaxis behaviors across different dosage levels of isotretinoin. Both doses of isotretinoin 125 and 250 mg/kg result in significant alterations in serotonin levels. Mice treated with isotretinoin at 125 mg/kg exhibit a decrease in serotonin levels compared to the control group. Both doses of isotretinoin result in significant changes in acetylcholine levels. Isotretinoin at 125 mg/kg shows a slight increase in acetylcholine. The data indicate that there is a significant increase in COMT enzyme. Histopathological study in the brain revealed that 125 mg/kg of isotritinoin showed mild vacuolization, blood vessel

congestion, and mild perivascular edema. High-dose 250 mg/kg recorded vacuolization, gliosis, blood vessel congestion, hemorrhage, and satellitosis.

Conclusion: high oral dosages of isotretinoin influence animal neurobehavioral behavior because of its effect on brain tissue, as evidenced by its effects on serotonin, acetylcholine, and the COMT enzyme.

Keywords: isotretinoin, neurobehavior, neurotransmitters, COMT enzyme.

Introduction

Isotretinoin is prescribed to treat Schnauzer comedone syndrome, ichthyosis, feline acne, sebaceous adenitis, epithelial lymphoma, keratoacanthoma, sebaceous gland hyperplasia, and adenomas (Koch *et al.,* 2010).

Other names for this medicine include Accutane®, Claravis®, Sotret®, isotretinoin, and retinoid. Retinoids are a family of vitamin A-derived chemicals that belong to the nuclear receptor superfamily and regulate gene transcription (Gudas, 2012). . They conduct numerous roles. This signaling molecule binds to particular retinoic acid receptors in the brain, including glucocorticoid and thyroid hormone receptors (Gudas, 2012).

Research into retinoic acid in the central nervous system has concentrated on brain development, spurred in part by the discovery that isotretinoin, an isomer of retinoic acid used in therapy (JIMENEZ *et al*., 2017). Recent research reveals that retinoic acid may alter the adult brain; animal studies have shown that isotretinoin administration causes behavioral abnormalities as well as inhibition of neurogenesis in the hippocampus. Isotretinoin inhibits fat cell growth and stimulates apoptosis, which reduces sebaceous gland output and size. It inhibits the migration of multinucleated white blood cells to the skin (JIMENEZ *et al*., 2017).

Isotretinoin affects a collection of nuclear receptors that control the expression of several gene receptors in the skin. Retinoic acid is derived from vitamin A and regulates cell proliferation and differentiation in several organs of the body, including bones, blood vessels, the heart, and immunity (Szymański *et al*., 2020). Recent research has highlighted the effects of isotretinoin on neural health, particularly concerning nerve cell proliferation, differentiation, and adaptability. Elevated or reduced isotretinoin levels significantly impact these processes (Melnik, 2019). Studies in companion animals, specifically dogs and cats, indicate psychological changes associated with isotretinoin use, including increased anxiety, aggression, and notable behavioral shifts (Camps et al., 2019).

In experimental studies on rodents, evidence also suggests that isotretinoin influences mood and behavior. For example, O'Reilly et al. (2006) demonstrated that a dosage of 1 mg/kg/day over six weeks in rats induced depression-like symptoms, as observed in behavioral tests like the forced swim test and the tail suspension test, where affected animals exhibited decreased activity. Additionally, isotretinoin has been associated with memory and learning impairments. Bremner (2021) reported hippocampal shrinkage, which aligns with findings on isotretinoin's negative impact on cognitive functions.

Given the abundance of retinoid receptors in various brain regions, isotretinoin has been implicated in inhibited brain growth across several animal models. However, limited studies have explored its specific neurobehavioral effects on adult animals, particularly regarding learning, memory, and anxiety. This study aims to fill this gap by examining the impact of isotretinoin on these neurobehavioral mechanisms in adult models, thereby addressing a critical void in current literature.The objective of this study is to comprehensively assess the effects of isotretinoin on neurobehavioral functions, including learning, memory, and anxiety in adult animal models.

Materials and Methods

Materials used in the study

Isotretinoin comes in the form of oil capsules form Ajanta Company, Jordan. The dose was determined based on the animal's weight and the dose delivered orally by gavage needle).

Ethical approval: All ethical approvals were acquired from College of Vet- Medicine, University of Mosul 11/15/2023,Ref: M.VET.2023.036

Animals

Male mice measuring 25-30 grams, 2-month age, were raised in laboratory circumstances that were temperature and humidity controlled, and they were kept and raised in dedicated cages inside the animal home.

Diagnostic kits

All kits using ELIZA for measuring

• A kit for measuring ACETYL CHOLINE from Elabscience Company

•A kit for measuring SEROTONIN 5-HT from the company Elabscience Company

• A kit to measure (COMT) catechol-O-methyltransferase from the company Elabscience Company, Lot Number: E202311045

Experiment design

A. LD50 experiment

To determine the median lethal dose (LD50), an initial dose is administered to a single animal based on preliminary experiments. The survival or death of the animal is observed after 24 hours. If the animal dies, the dose is decreased by a fixed amount; if the animal survives, the dose is increased by the same fixed amount. This process is repeated until a change occurs, usually indicated by a reversal in outcome (i.e., a live animal followed by a dead one, or vice versa).

After this turning point, observations are recorded for three additional animals over three days. The results are noted in a binary format, where "X" represents the death of the animal, and "0" indicates survival. The final LD50 value is calculated according to a table described in Dixon's 1980 study.

A wide range of dosages were employed to calculate the LD50 . The LD50 is calculated using a formula that includes the first orally provided dose (Xf), a coefficient (K) indicating the rise or decrease in dose, and the last orally delivered dose. Based on the numbers supplied, the first orally administered dose (Xf) is 4000 mg/kg, the coefficient (K) is 0.701, and the dose increase or decrease is 1200 mg/kg.

Substituting these values into the LD50 formula, we obtain:

LD50 = Xf + Kd

B- Evaluation of various doses of isotretinoin on nervous system:

in this investigation, 15 mice were placed into three groups: the first was a control group that received no therapy, the second group received 125 mg/kg isotretinoin, and the third group received 250 mg/kg by orally dosage.

Dose Selection

The doses of 125 and 250 mg/kg of isotretinoin were selected based on several factors. First, the selection was guided by the LD50 value of 4841.2 mg/kg in rats, which indicates the median lethal dose. Choosing doses well below this level minimizes the risk of acute toxicity and ensures safety.

Secondly, these doses were informed by previous studies on the effects of isotretinoin. These studies demonstrated notable neurobehavioral effects at moderate doses lower than the LD50. Thus, the 125 and 250 mg/kg doses allow for observing behavioral and neurological impacts within a safe range, while also revealing any significant sub-acute toxicity effects relevant to the study. The therapy session lasted 14 days .

Neurobehavioral assessments were taken following the treatment period, including:

- The open field test done by counting the number of rearing and squares that the mouse passes within an open field box daimeter 40 cm x 40 cm x 30 cm (length x width x height) (Gould *et al,*, 2009).

- The light and darktest makes use of a specific equipment in the shape of a box divided into two rooms: dark and light. After placing the mouse inside the box for 3 minutes, the time spent by each mouse inside each chamber is measured, and the percentage remaining within the dark room is determined using the equation dark time = (dark time) /(total time) * 100 (Kulesskaya and Voikar, 2014).

- The negative geotaxis test involves placing the animal on a device with a sloping surface at a 45-degree angle, and then calculating the time, it takes the animal to turn and change direction within seconds (Kulesskaya and Voikar, 2014).

- The pocking test involves utilizing a device in the shape of a perforated surface with numerous holes the size of the mouse's head. The mouse is placed on this surface and left for three minutes. The number of holes in which the mouse places its head during a period is counted (Hurst and West, 2010).

After the behavioral and neurological tests were finished, the mice were given ether anesthesia so that blood could be drawn from them. The blood was then placed in glass tubes containing the anticoagulant EDTA in order to separate the plasma for future biochemical assays. The brain was also removed and stored in clean containers containing 10% neutral formalin.

The enzyme catechol-O-methyltransferase (COMT) is crucial in the breakdown of catecholamines such as dopamine, epinephrine, and norepinephrine. COMT works by transferring a methyl group to catecholamines, thereby inactivating them and playing an important role in regulating mood, cognition, and stress responses.

Statistically analysis

The statistical interpretation of these findings entails determining the significance of the observed differences, which are indicated in the tables. Statistical testing using the SPSS program for analysis of variance (ANOVA) and **post-hoc comparisons with the Tukey HSD test** revealed significant differences at $p \leq 0.05$.

Results

The provided data outlines the oral LD50 (lethal dose for 50% of the test animal) for isotretinoin. The LD50 value is crucial in toxicology as it indicates the dosage at which a substance becomes lethal to half of the test animal. In this case, the LD50 for isotretinoin is calculated to be 4841.2 mg/kg (Table 1).

LD50 = Xf + Kd

4000 + (0.701 * 1200) = 4841.2 mg\kg

The neurobehavioral measurements of mice treated with isotretinoin for 14 consecutive days reveal significant effects on various parameters compared to the control group. The data presented in Table 2 illustrate changes in open field activity, time spent in dark areas, and negative geotaxis behaviors across different dosage levels of isotretinoin.

Starting with open field activity, both doses of isotretinoin (125 mg/kg and 250 mg/kg) show alterations compared to the control group. Mice treated with 125 mg/kg exhibit an increase in locomotor activity, as indicated by a increase number of crossings and rearing. The higher dose of isotretinoin (250 mg/kg) exacerbates this effect, resulting in a more pronounced increase in both crossing and rearing activities compared to both the control and lower dose groups.

Regarding the percentage of time spent in dark areas, there is a notable difference between the control group and the isotretinoin-treated groups. Mice administered with both doses of isotretinoin spend significantly less time in dark areas compared to the control group.

In the negative geotaxis test, significant differences are observed between the control group and the isotretinoin-treated groups. Mice receiving isotretinoin, particularly at the higher dose of 250 mg/kg, exhibit impaired performance in negative geotaxis and pocking number compared to the control and lower dose groups (Table 2).

Table 3 recorded compared to the control group, both doses of isotretinoin (125 mg/kg and 250 mg/kg) result in significant decreased in serotonin levels. Also Both doses of isotretinoin (125 and 250 mg/kg) result in significant increase in acetylcholine levels. Mice treated with isotretinoin at 125 mg/kg show a slight increase in acetylcholine levels compared to the control group. In addition to that, data indicate that there are significant increase in COMT levels between the control group and the isotretinoin-treated groups at either dose (125 mg/kg or 250 mg/kg).

Histopathological study Results

Figure 1 displays histological sections of the brains of mice from different experimental groups. In panels A and B, representing the control group, normal architecture of neurons (indicated by arrows), glial cells (thick arrows), and blood vessels (arrowheads) is observed. These sections exhibit no significant abnormalities.

In panels C and D, corresponding to the low dose (125 mg) of isotritinoin group, mild vacuolization (arrows), blood vessel congestion (thick arrows), and mild perivascular edema (arrowheads) are evident. These changes suggest some degree of tissue alteration, although they are relatively minor compared to the control group.

In panels E and F, representing the high dose of isotritinoin (250 mg) group, more pronounced histological changes are observed. Vacuoles (indicated by arrows) gliosis (pointed out by arrows) congested and hemorrhaged blood vessels (marked by arrowheads) and satellitosis (shown with curved arrows) are clearly visible. These changes suggest damage, to the tissue and an inflammatory response is underway.

Both low and high doses of isotretinoin seem to cause alterations in the brain tissue of mice with severe changes observed at higher doses. The figures in the panel are magnified at 100X providing a view of the tissue structure while those in the right panel are magnified at 400X offering a closer look at cellular details.

The histological sections have been stained with hematoxylin and eosin (H&E) to visualize shapes and tissue structures. These histopathological findings offer insights into the neurotoxic effects of isotretinoin at varying doses underscoring the need for further research on its safety profile and possible impacts, on the central nervous system.

A specialized pathologist with expertise in brain tissue analysis was relied upon to ensure the accuracy and reliability of the results. The pathologist evaluated the tissue samples using advanced methods and standardized criteria, allowing for a comprehensive analysis of histopathological changes. This

evaluation included examining cellular structures, assessing the degree of damage, and monitoring changes that may indicate the effects of treatment or toxicity.

Discussion

This research examined isotretinoin's effects on animal behavior, motor activity, neurotransmitter interactions, and brain tissue. The LD50 was found to be 4841.2 mg/kg, indicating toxicity risks at high doses. Neurobehavioral tests showed that isotretinoin increased motor activity, such as standing time and squares crossed, suggesting brain effects that manifest as anxiety (Gould *et al*., 2009.).The light and darkness experiment also indicated that the mouse preferred staying in the light, than the dark indicating stress experienced by the animals possibly due to nerve receptor stimulation in the brain. Similarly, findings from the negative geotaxis' test showed that it took longer for the animal to turn around suggesting impairment in its vestibular brain functions. The brain plays a role, in maintaining balance and a decreased interest level implies that the animals may not be fully aware of their environment (Kulesskaya and Voikar, 2014).

In this study, we explored how certain brain neurotransmitters may be influencing behavioral changes. The rise in anxiety and tension seen in the animals could be due to disturbances in levels of neurotransmitters like Ach and serotonin (Hurst and West, 2010). Serotonin, a neurotransmitter that

impacts mood, cognition and behavior has been linked to mental health conditions like sadness and anxiety. The decrease in levels post isotretinoin treatment raises concerns about mood related side effects from the therapy (Dopheide and Morgan, 2008). These findings highlight isotretinoin's influence on neuropsychiatric side effects, including altered acetylcholine levels post-treatment (Kontaxakis *et al.,* 2009). Acetylcholine is crucial for neurotransmission, muscle movement, and cognition. The increase in acetylcholine observed may indicate effects on cholinergic mechanisms (Bacqué-Cazenave *et al*., 2020), though the exact process—whether through production, release, or breakdown in the peripheral nervous system—remains unclear (Ding *et al.,* 2023). Patients on isotretinoin should be monitored for side effects such as muscle weakness, digestive issues, or cognitive concerns (Rose and Goldberg, 2013). Both doses of isotretinoin significantly raised COMT levels, an enzyme that metabolizes neurotransmitters like dopamine, serotonin, norepinephrine, and epinephrine, which may explain the serotonin decline observed in this study. COMT regulation affects mood, stress, and cognitive function (Brandt and Flurie, 2020), and altered COMT activity is associated with conditions like schizophrenia, depression, and Parkinson's disease (Clayton *et al.,* 2020). Histopathological analysis showed dose-dependent effects on brain tissue, with findings in line with prior studies showing isotretinoin's impact on nerve cell development and repair (Balch *et al*., 2008).

Isotretinoin has been linked to delays in brain development, affecting mental and nervous system growth (Meloto *et al.,* 2015). Research shows that isotretinoin impairs neuron growth in the hippocampus—crucial for memory and learning—by impacting gene activity that regulates nerve cell growth, survival, and repair, accelerating neuron death in this area (Bremner *et al*., 2011). As an anti-inflammatory, isotretinoin reduces skin inflammation by inhibiting leukocyte migration and likely functions through retinoic acid receptor activation, affecting gene expression (Clark *et al.,* 2020; Isoherranen and Zhong, 2019). Retinoic acid from vitamin A is essential for cell differentiation across various systems, including the hippocampus, which relies on neuronal plasticity and neurogenesis for memory formation (Dabrowska and Thaul, 2018). Histopathology confirms isotretinoin's dose-dependent toxicity in brain tissue, aligning with previous findings showing restricted nerve cell formation and repair (Nurjanti, 2019).

In addition to damage to synapses, which are the communication points between nerve cells, there are brain development issues such as delayed mental and nervous development (Huang *et al.*, 2014). Researchers also discovered that isotretinoin inhibits the development of new neurons in the hippocampus, a brain region critical for memory and teach (Clark *et al.,* 2020).

Isotretinoin alters the expression of a number of genes in nerve cells, including those involved in nerve cell growth and death, thus slowing the repair of damaged nerve cells in this brain region (Ormerod, 2021; Moini, and Piran, 2020).

Researchers also discovered that isotretinoin causes nerve cell loss in the hippocampus (Al-Abdaly *et al*., 2023; Khodabakhshi Rad *et al.,* 2023).

Our research, into the effects on behavior and biochemistry caused by isotretinoin matches what other studies have found in the past – that isotretinoin can affect neurotransmitter levels and brain function. Previous studies conducted by Bremner *et al.,* 2005 and O'Reilly *et al.,* 2008 have also reported changes, in pathways and behavioral changes after administering isotretinoin. This helps explain why we saw serotonin levels in mice given a dose of 125 mg/kg in our study. The disturbance of this chemical messenger is acknowledged as an element, in alterations to patterns; research has connected these shifts to emotional disorders and cognitive limitations while also heightening the response, to stress.

However there are differences, from studies. For instance past investigations mostly concentrated upon low level application of isotretinoin whereas our study assessed sudden dosages. Our discoveries, particularly concerning increased acetylcholine amounts. Intensified COMT enzyme function are fresh and indicate that immediate isotretinoin exposure, at levels could provoke distinctive biochemical reactions perhaps by means of increased oxidative stress or acetylcholine pathway adjustment. These

discoveries enhance our knowledge of the impacts of isotretinoin. Emphasize the necessity, for more exploration, into dose related neurochemical alterations.

Addressing experiments presents difficulties and suggestions to consider moving forward when dealing with the careful monitoring required for high doses of isotretinoin because of potential toxicity risks involved in the process.A notable challenge arises from the varying neurobehavior exhibited by mice which could be influenced by factors affecting the outcomes of experiments conducted .

Future research should consider investigating the impact of doses over time to mimic real world usage patterns in settings. It would be beneficial to study the long term effects of isotretinoin, on neurotransmitters such as serotonin and acetylcholine as COMT in diverse populations across different age groups and genders. Additionally exploring the benefits of using substances like antioxidants or enzyme inhibitors to mitigate the neurotoxic effects of isotretinoin could be valuable, for further research .

Conclusion

We conclude that oral over dose dosages of isotretinoin influence in animal neurobehavioral because of its effect on brain tissue, as evidenced by its effects on serotonin, acetylcholine, and the COMT enzyme.

Acknowledgment: To vet-medicine college in university of Mosul.

Conflicted interest: None.

References

Bremner, J.D., Fani, N., Ashraf, A., Votaw, J.R., Brummer, M.E., Cummins, T., Vaccarino, V., Goodman, M.M., Reed, L., Siddiq, S. and Nemeroff, C.B., 2005. Functional brain imaging alterations in acne patients treated with isotretinoin. American Journal of Psychiatry, 162(5), pp.983-991. PMid:15863802

O'Reilly, K., Bailey, S.J. and Lane, M.A., 2008. Retinoid-mediated regulation of mood: possible cellular mechanisms. Experimental biology and medicine, 233(3), pp.251-258. PMid:18296731

Camps, T., Amat, M. and Manteca, X., 2019. A review of medical conditions and behavioral problems in dogs and cats. Animals, 9(12), p.1133. https://doi.org/10.3390/ani9121133 PMid:31842492 PMCid:PMC6941081

Koch, S.N., Torres, S.M. and Plumb, D.C., 2012. Canine and feline dermatology drug handbook. John Wiley & Sons. https://doi.org/10.1002/9781118704745

Gudas, L.J., 2012. Emerging roles for retinoids in regeneration and differentiation in normal and disease states. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1821(1), pp.213-221.https://doi.org/10.1016/j.bbalip.2011.08.002

PMid:21855651 PMCid:PMC3237723

JIMENEZ, R.E., HIEKEN, T.J., PETERS, M.S. and VISSCHER, D.W., 2017. Pagetneoplasticbetween disease (PD) is histologically defined as the presence of. The Breast: Comprehensive Management of Benign and Malignant Diseases, p.169.

https://doi.org/10.1016/B978-0-323-35955-9.00012-X

Szymański, Ł., Skopek, R., Palusińska, M., Schenk, T., Stengel, S., Lewicki, S., Kraj, L., Kamiński, P. and Zelent, A., 2020. Retinoic acid and its derivatives in skin. *Cells*, 9(12), p.2660.https://doi.org/10.3390/cells9122660.PMid:33322246 PMCid:PMC7764495

Melnik, B.C., 2019. Mechanism of action of isotretinoin. In: *Retinoids in Dermatology*. CRC Press, pp.13-25.https://doi.org/10.1201/9780429456732-4

O'Reilly, K.C., Shumake, J., Gonzalez-Lima, F., Lane, M.A. and Bailey, S.J., 2006. Chronic administration of 13-cis-retinoic acid increases depression-related behavior in mice. *Neuropsychopharmacology*, 31(9), pp.1919-1927.https://doi.org/10.1038/sj.npp.1300998.PMid:16395305

Bremner, J.D., 2021. Isotretinoin and neuropsychiatric side effects: continued vigilance is needed. *Journal of Affective Disorders Reports*, 6, p.250230.

https://doi.org/10.1016/j.jadr.2021.100230. PMid:37168254 PMCid:PMC10168661

Dixon, W. 1980. Effect analysis of expermintal observation. Annu. Rev. Pharm. Toxicol., 20 :441-426.

Gould, T.D., Dao, D.T. and Kovacsics, C.E., 2009. The open field test. In: *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests*. pp.1-20.https://doi.org/10.1007/978-1-60761-303-9_1

Kulesskaya, N. and Voikar, V., 2014. Assessment of mouse anxiety-like behavior in the light-dark box and open-field arena: role of equipment and procedure. *Physiology & Behavior*, 133, pp.30-38.https://doi.org/10.1016/j.physbeh.2014.05.006.PMid:24832050

Hurst, J.L. and West, R.S., 2010. Taming anxiety in laboratory mice. *Nature Methods*, 7(10), pp.825-826.https://doi.org/10.1038/nmeth.1500.PMid:20835246

Dopheide, M.M. and Morgan, R.E., 2008. Isotretinoin (13-cis-retinoic acid) alters learning and memory, but not anxiety-like behavior, in the adult rat. *Pharmacology Biochemistry and Behavior*, 91(2), pp.243-251https://doi.org/10.1016/j.pbb.2008.08.009.PMid:18760297

Kontaxakis, V.P., Skourides, D., Ferentinos, P., Havaki-Kontaxaki, B.J. and Papadimitriou, G.N., 2009. Isotretinoin and psychopathology: a review. *Annals of General Psychiatry*, 8, pp.1-8. https://doi.org/10.1186/1744-859X-8-2.PMid:19154613 PMCid:PMC2637283

Bacqué-Cazenave, J., Bharatiya, R., Barrière, G., Delbecque, J.P., Bouguiyoud, N., Di Giovanni, G., Cattaert, D. and De Deurwaerdère, P., 2020. Serotonin in animal cognition and behavior. *International Journal of Molecular Sciences*, 21(5), p.1649.

https://doi.org/10.3390/ijms21051649. PMid:32121267 PMCid:PMC7084567

Ding, R.L., Zheng, Y. and Bu, J., 2023. Physiological and psychological effects of isotretinoin in the treatment of patients with acne: a narrative review. *Clinical, Cosmetic and Investigational Dermatology*, pp.1843-1854.https://doi.org/10.2147/CCID.S416267.PMid:37483471 PMCid:PMC10362870

Rose, A.E. and Goldberg, D.J., 2013. Safety and efficacy of intradermal injection of botulinum toxin for the treatment of oily skin. *Dermatologic Surgery*, 39(3 pt1), pp.443-44/. https://doi.org/10.1111/dsu.12097.PMid:23293895

Brandt, N. and Flurie, R., 2020. Acetylcholine. In: *Encyclopedia of Behavioral Medicine*. pp.18-19.https://doi.org/10.1007/978-3-030-39903-0_1351

Clayton, R.W., Langan, E.A., Ansell, D.M., de Vos, I.J., Göbel, K., Schneider, M.R., Picardo, M., Lim, X., van Steensel, M.A. and Paus, R., 2020. Neuroendocrinology and neurobiology of sebaceous glands. *Biological Reviews*, 95(3), pp.592-624.

https://doi.org/10.1111/brv.12579. PMid:31970855

Balch, J.F., Stengler, M. and Young-Balch, R., 2008. *Prescription for Drug Alternatives: All-Natural Options for Better Health without the Side Effects*. Turner Publishing Company.

Meloto, C.B., Segall, S.K., Smith, S., Parisien, M., Shabalina, S.A., Rizzatti-Barbosa, C.M., Gauthier, J., Tsao, D., Convertino, M., Piltonen, M.H. and Slade, G.D., 2015. COMT gene locus: new functional variants. *Pain*, 156(10), pp.2072-2083.

https://doi.org/10.1097/j.pain.0000000000000273.PMid:26207649 PMCid:PMC4748386

Bremner, J.D., Shearer, K.D. and McCaffery, P.J., 2011. Retinoic acid and affective disorders: the evidence for an association. *The Journal of Clinical Psychiatry*, 72(1), p.18228.

Clark, J.N., Whiting, A. and McCaffery, P., 2020. Retinoic acid receptor-targeted drugs in neurodegenerative disease. *Expert Opinion on Drug Metabolism & Toxicology*, 16(11), pp.1097-1108. https://doi.org/10.1080/17425255.2020.1811232.PMid:32799572

Isoherranen, N. and Zhong, G., 2019. Biochemical and physiological importance of the CYP26 retinoic acid hydroxylases. Pharmacology & therapeutics, 204, p.107400.

https://doi.org/10.1016/j.pharmthera.2019.107400.PMid:31419517 PMCid:PMC6881548

Dabrowska, A. and Thaul, S., 2018. How FDA approves drugs and regulates their safety and effectiveness. Washington: Congressional Research Service, pp.1-25.

Nurjanti, L., 2019. The role of immunological reaction and pro-inflammatory mediators in acne vulgaris etiopathogenesis, applications in dermatology practice. *International Journal of Clinical& Experimental Dermatology*, 4(1), pp.1-44.https://doi.org/10.33140/IJCED.04.01.01

Huang, P., Chandra, V. and Rastinejad, F., 2014. Retinoic acid actions through mammalian nuclear receptors. *Chemical Reviews*, 114(1), pp.233-254.https://doi.org/10.1021/cr400161b

PMid:24308533 PMCid:PMC3931982

Clark, J.N., Whiting, A. and McCaffery, P., 2020. Retinoic acid receptor-targeted drugs in neurodegenerative disease. *Expert Opinion on Drug Metabolism & Toxicology*, 16(11), pp.1097-1108.https://doi.org/10.1080/17425255.2020.1811232.PMid:32799572

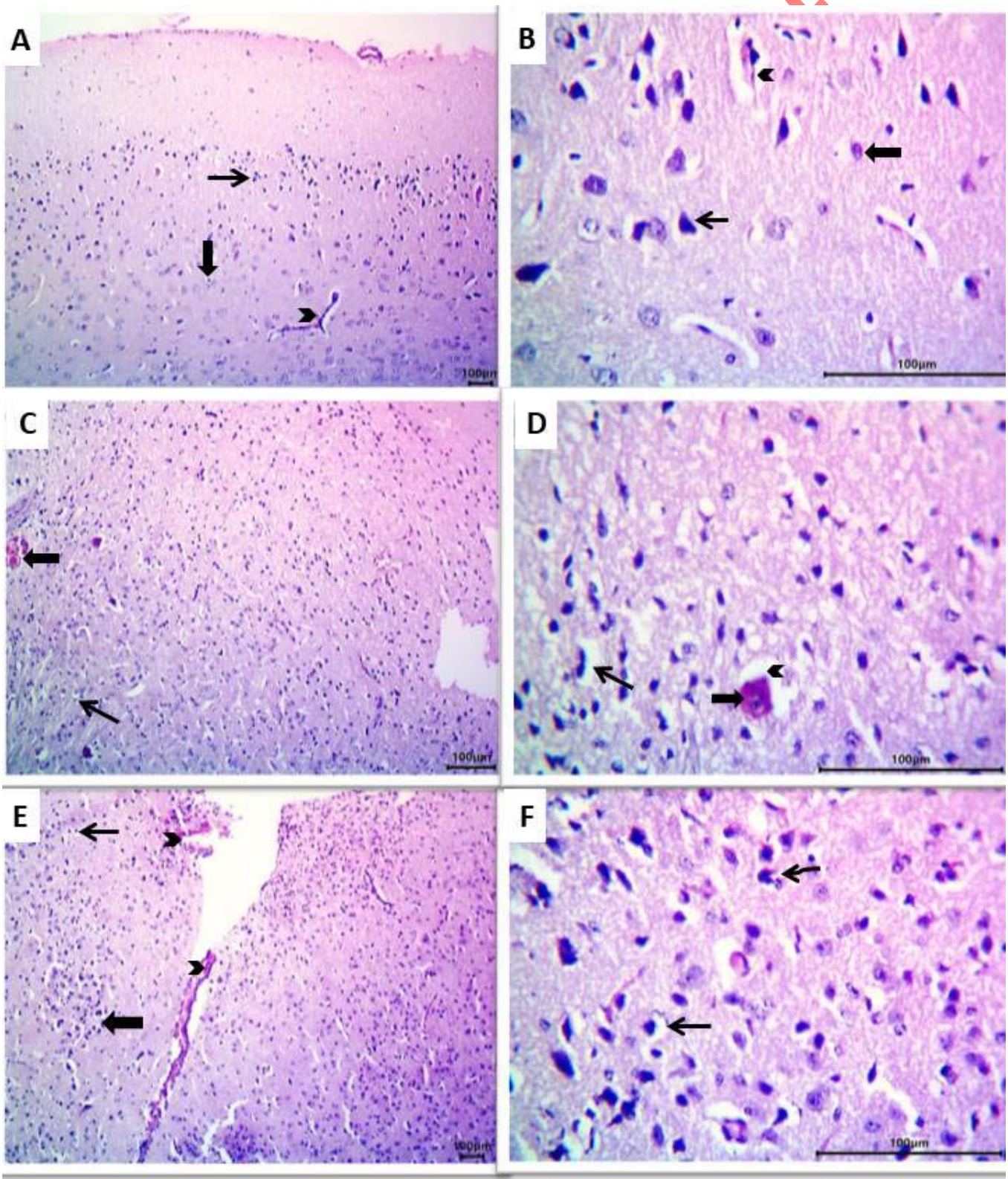
Moini, J. and Piran, P., 2020. *Functional and Clinical Neuroanatomy: A Guide for Health Care Professionals*. Academic Press.

Ormerod, A.D., Thind, C.K., Rice, S.A., Reid, I.C., Williams, J.H. and McCaffery, P.J., 2012. Influence of isotretinoin on hippocampal-based learning in human subjects. *Psychopharmacology*, 221, pp.667-674.https://doi.org/10.1007/s00213-011-2611-y

PMid:22193726 PMCid:PMC3360864

Al-Abdaly, Y., Alfathi, M. and Al-Mahmood, S., 2023. Comparison of azithromycin toxicity in chickens and quails. Iranian Journal of Veterinary Medicine, 17(4), pp.321-332.https://doi.org/10.32598/IJVM.17.4.1005354

Khodabakhshi Rad, A., Kazemi Mehrjerdi, H., Pedram, M. S., Azizzadeh, M., Amanollahi, S. 2023. 'Clinical Evaluation of the Effect of Methylprednisolone Sodium Succinate and Meloxicam in Experimental Acute Spinal Cord Injury', Iranian Journal of Veterinary Medicine, 17(2), pp. 129-138. doi: 10.32598/ijvm.17.2.1005246


A
B
C
D
E
F
100µm