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NEURO-PROTECTIVE EFFECT OF BEE AND DATE PALM POLLENS AGAINST IBUPROFEN TOXICITY IN MALE ALBINO RATS

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ABSTRACT

Non-steroidal anti-inflammatory medications (NSAIDs), like Ibuprofen (IBP), are produced in enormous quantities throughout the world to treat a wide range of diseases. Bee Pollen (BP) is a natural antioxidant. Bee products are utilized in modern medicine to treat inflammatory diseases. Date Palm Pollen (DPP) is an organic substance produced by date palm trees that symbolizes the male reproductive cells of palm flowers. It is an excellent choice for diet because of its high nutritional content and abundance of phenols and flavonoids, which act as natural antioxidants. This study aimed to validate the protective impact of the combined pollen from date palms and bees on ibuprofen-induced brain toxicity. Forty-two (42) albino rats have been used for this study. Animals were treated with BP (100 mg/kg b. wt.) and DPP (100 mg/kg b. wt.) daily for one month, followed by IBP (60 mg/kg b. wt.) both individually and in combination for thirty days in a row. This research showed severe alterations in the structure of the brain regions induced by IBP. These alterations were improved by the combination of BP and DPP. This research showed that BP and DPP (as antioxidants) have the power to restore the architecture of the brain tissue and stimulate anti-oxidative defenses, which may protect against brain toxicity brought on by IBP.

Keywords: Brain toxicity, non-steroidal, anti-inflammatory drugs, bee pollen, date palm pollen, albino rats.

INTRODUCTION

Non-steroidal anti-inflammatory medications (NSAIDs), including Paracetamol, Diclofenac sodium and IBP are still used to treat inflammatory illnesses, such as rheumatological and menstrual disorders, toothache and dysmenorrhea (Chen *et al.,* 2005; Mahalakshmi *et a1.,* 2010). IBP is a well-known NSAID that is usually prescribed to relieve pain, fever and inflammation. It works by reducing prostaglandin synthesis by cyclogenase activity suppression. (Ha and Peak, 2021). IBP could control the inflammatory process and lower the amount of proinflammatory cytokines produced (Albertini *et al.,* 2021). The administration of IBP has been shown to shield neurons from glutamate toxicity (Ramires *et al*., 2023) Also, it was stated that IBP, as an anti-inflammatory drug, reduces neurological pain

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and post-surgical pain (Jan-Roblero and Gruz-Maya, 2013). However, it was stated that IBP as NSAIDs with antipyretic and analgesic properties induced histopathological changes in the architecture of vital organs such as the liver (Hasnawi *et al.,* 2022, Satvati *et al.,* 2022, Priyadarshinee *et al.,* 2023), kidney (Hadi and Abood, 2022), spleen (Shafi and Shabir, 2019), stomach (Kholmurodovich, 2022; Lisma and Stepchenko, 2022), testes (Sharma *et al.,* 2020) and nervous system (Sibiya *et al.,* 2023) in the experimental animals.

Even with the development of new synthetic medications with antioxidant qualities, natural products still include potent antioxidant molecules that are still utilized to stop oxidative stress-related damage and toxicity. Hence, many studies have been conducted on natural products rich in phytochemical components such as phenols and flavonoids due to their antioxidant biological properties (Carpes *et al.,*2007; Al-Orf *et al.,*2012).

Bee pollen (BP) is a naturally occurring substance that has been shown to have potent antioxidant properties, since it contains components with phytochemical functions, such as flavonoids, phenolamides and phenolic acids (Aylanc, V. *et al.,* 2021; Wu *et al.,* 2019). It is known as floral life and collected by honeybee workers from the plants' anthers. Bees combine floral pollen with salivary gland secretions, honey and nectar. Bee pollen is the final product (Thakur and Nanda, 2020), then aggregates in granules form and compacted into a pellet (Saravedra *et al.,* 2013), in the hive entrance and used as a major source of feeding for bee growth (Xue *et al.,* 2012). Because bee pollen has so many nutritional components, it is considered an important dietary supplement. Many cosmetics, health food items, and contemporary medical applications use bee pollen (Aylanc, V. *et al.,* 2021). Several studies have indicated that bee pollen can be used as an anti-inflammatory, antioxidant, antibacterial and have immune-boosting characteristics, aid in the healing of persistent diseases, delaying aging, reducing cholesterol, and regulating digestive processes (Li *et al*., 2018, Thaker and Nanda, 2020). So, it plays a vital role in human health (EL-Seedi *et al.,* 2022) and experimental animals (Kędzierski *et al.,* 2020).

Date palm pollen (DPP) is an important natural antioxidant of plant origin. This powdered male reproductive system is made from the flowers of the *Phoenix dactylifera L.* palm trees and has been used as a supplement to food, especially to increase fertility in both male and female humans (Salhi *et al.,* 2024). It is an image of the male reproductive cells found in palm blossoms, which appear as fine dust-like grains and normally waxy white (Halbritter *et al.,* 2018). It is called by ancient Egyptian and Chinese “fountain of youth” (Kroyer and Hegedus, 2001).

DPP's abundance of minerals, vitamins and amino acids makes it a great nutritional food supplement for both people and experimental animals (Salmani *et al*., 2022). Because of many phytochemicals, including biologically volatile unsaturated fatty acids, phenols and flavonoids, which are significant antioxidants, it might be considered a functional food (Jahromi *et al*., 2022). DPP could act as an antioxidant preventing free radicals’ generation in the vital organs exposed to xenobiotics (Blade *et al.*, 2016).

Based on the antioxidant and protective effects of BP and DPP as natural antioxidants on the brain structure, the present study aimed to illustrate the potential effect of each BP and DPP and their combination against IBP toxicity on the brain structure.

The present study bears originality regarding the existence of any previous studies using the combination of BP and DPP for IBP toxicity on the architecture of the brain in experimental animals. The results of the present study could provide new targets of BP and DPP for improving the toxicity of IBP on the biological systems that were not fully studied.

MATERIAL AND METHODS

Chemicals:

Ibuprofen (IBP) is 2-[4-(2-methylpropyl) Phenyl] Propanoic acid was purchased from Labo Chem (India) with a purity of 95%. It was powder, dissolved in distilled water, and administered as aqueous suspension orally.

Bee Pollen (BP) was in the form of dried granules obtained from a professional beekeeper in Sohag Governorate, Egypt. The granules were suspended in distilled water using a sonicator and supplemented orally as aqueous suspension.

Date Palm Pollen (DPP) grains were acquired from the Sohag University's, Faculty of Agriculture Farm in Sohag, Egypt. Then, they were dried, washed and blended. Thereafter they were kept in the refrigerator until use. On use, DPP is suspended in distilled water using a sonicator and administered as aqueous suspension orally.

Experimental animals and ethical approval:

The Institutional Animal Ethics Committee of the Faculty of Science at Sohag University in Sohag, Egypt, authorized the study (CSRE-34-24). The animal house of the Zoology Department of the Faculty of Science, Sohag University, Sohag, Egypt is the source of a male albino rat (*Rattus rattus*) weighing 230±5 grams. Water was available to all animals, and they were fed normal rat food at a temperature of 25±2 °C with a 12/12 h light/dark cycle.

Experimental design:

After being purchased from a licensed breeder, the rats were split randomly into seven separate groups of six rats. The animals were administered the treatments orally daily for one month with a pre-experimental period of two weeks for adaptation to the lab environment. Animals of the first group served as normal controls, while group II was treated with IBP at a dose of 60 mg/kg b. wt. (Gomaa, 2018). Group III was treated with BP (100 mg/kg b. wt.) (El-Sayed *et al.,* 2023a). Group IV was treated with DPP (100 mg/kg b. wt.) (El-Sayed *et al.,* 2023b). Group V was treated with BP before IBP, while Group VI was treated with DPP before IBP. Group VII was treated with combined BP and DPP along with IBP. The animals were observed daily in their cages for clinical signs. Mortality did not occur.

Histopathology:

Small representative pieces of brain tissue were collected in 10% neutral buffered formalin after washing well with physiological saline (NaCl 0.9%) to remove fat, connective tissue and blood, then plotted on the filter paper. The organ's specimen was prepared for histological examination and processed routinely paraffin embedding technique. Using a Leica microtome (Germany), embedded tissues were sectioned at a thickness of 5 µm, and then stained with hematoxylin and eosin (H&E) according to Drury and Wallington (1980). A light microscope (Zeiss, Primo star, Germany) was used to photograph the specimens for histological examination.

RESULTS

The histological examinations of the brain sections are represented in Plates (1-5). Plate (1A; B) showed that the cerebrum of the control group (G1) of rats in the grey matter showed normal histological structure of nerve cells (Plate 1A), and normal histological structure of hippocampus Ammon's horn (Plate 1B).

The cerebrum of rats, which was treated with IBP (G2) (60 mg/kg b. wt.), showed necrosis of nerve cells with darkly stained nuclei (Pyknosis of nuclei) and demyelination of some nerve fiber (plate 1C). Necrotic neurons were also seen in the hippocampus (Ammon's horn) (plate1D).

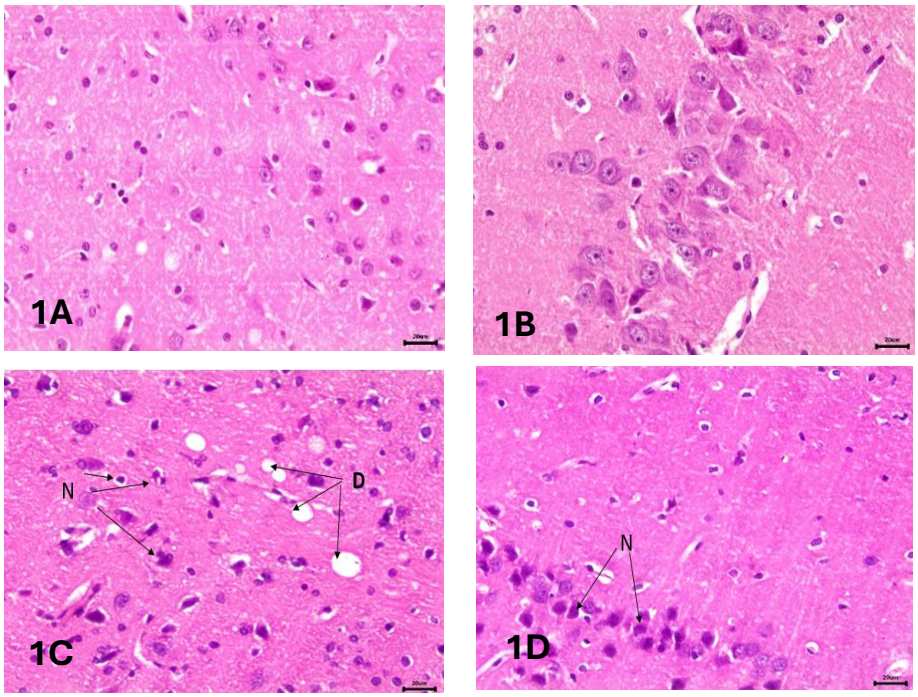

Plate 1A: Photomicrograph of cerebral gray matter (GM) from rats of the control group (G1) showing the normal histological structure of nerve cells (NC). **1B**: Photomicrograph of the hippocampus (H) from the control group (G1) showing the normal histological structure of Ammon's horn cells. **1C**: Photomicrograph of the cerebrum from Ibuprofen-treated group (G2) showing necrosis (N) of nerve cells with darkly stained (pyknotic) nuclei and demyelination (D). **1D**: Photomicrograph of hippocampus from Ibuprofen-treated group (G2) showing numerous necrotic (N) cells of the Ammon's horn cells. H&E stain. scale bar =20µm.

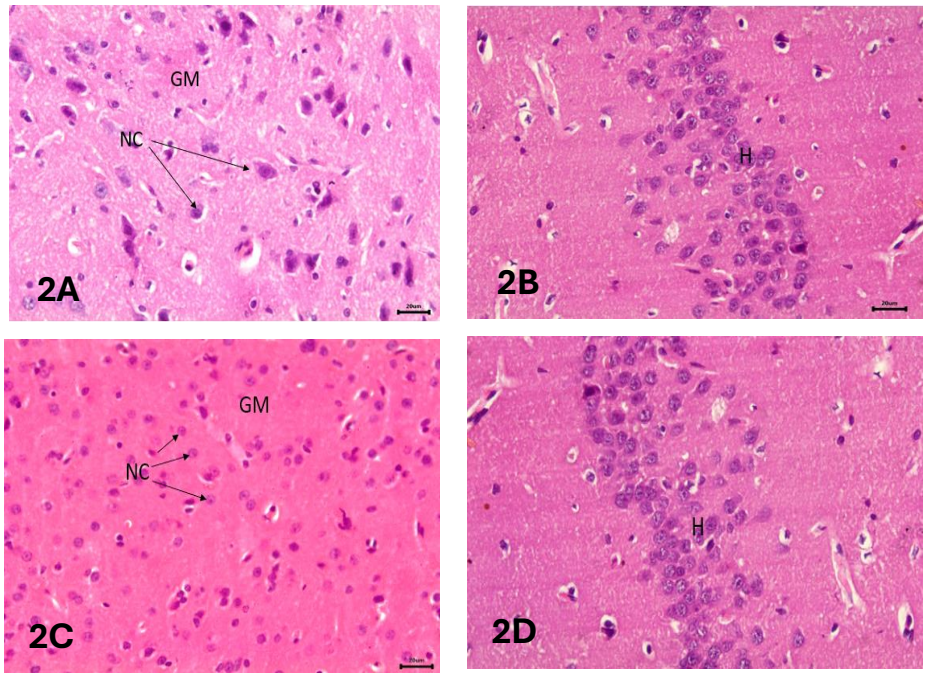

Plate 2: A) Photomicrograph of the cerebral gray matter (GM) of rats from the bee pollen-treated group (G3), showing the normal histological structure of nerve cells (NC). **B)** Photomicrograph of the hippocampus (H) from the BP-treated group (G3), showing the normal histological structure of Ammon's horn cells. **C)** Photomicrograph of the cerebral gray matter (GM) from the DPP-treated group (G4), showing the normal histological structure of nerve cells (NC). **D)** Photomicrograph of the hippocampus (H) from the DPP-treated group (G4), showing the normal histological structure of Ammon's horn cells. H&E stain. scale bar =20µm.

The cerebrum from rats which received BP (G3) (100 mg/kg b. wt.) showed the normal histological structure of nerve cells (plate 2A) in the cortex and hippocampus (Ammon's horn) (Plate 2B). In rats which were treated with DPP (100 mg/kg b. wt. (G4) also showed normal histological structure of nerve cells in the cortex (plate 2C) and hippocampus (Amman's horn) (plate 2D).

The cerebral cortex from the rats, which received BP with IBP (G5), showed neuronal necrosis (Plate 3A) and necrosis of a few nerve cells and perivascular edema in the hippocampus (plate 3B). The same result was obtained in the cerebral cortex and the hippocampus from rats which received DPP with IBP (G6) (plate 3 C; D). However, the rats which obtained a combined BP, DPP with IBP (G7) showed more or less normal cells with a few necrosis of some cells in the cortex and the hippocampus (plate 3E; F).

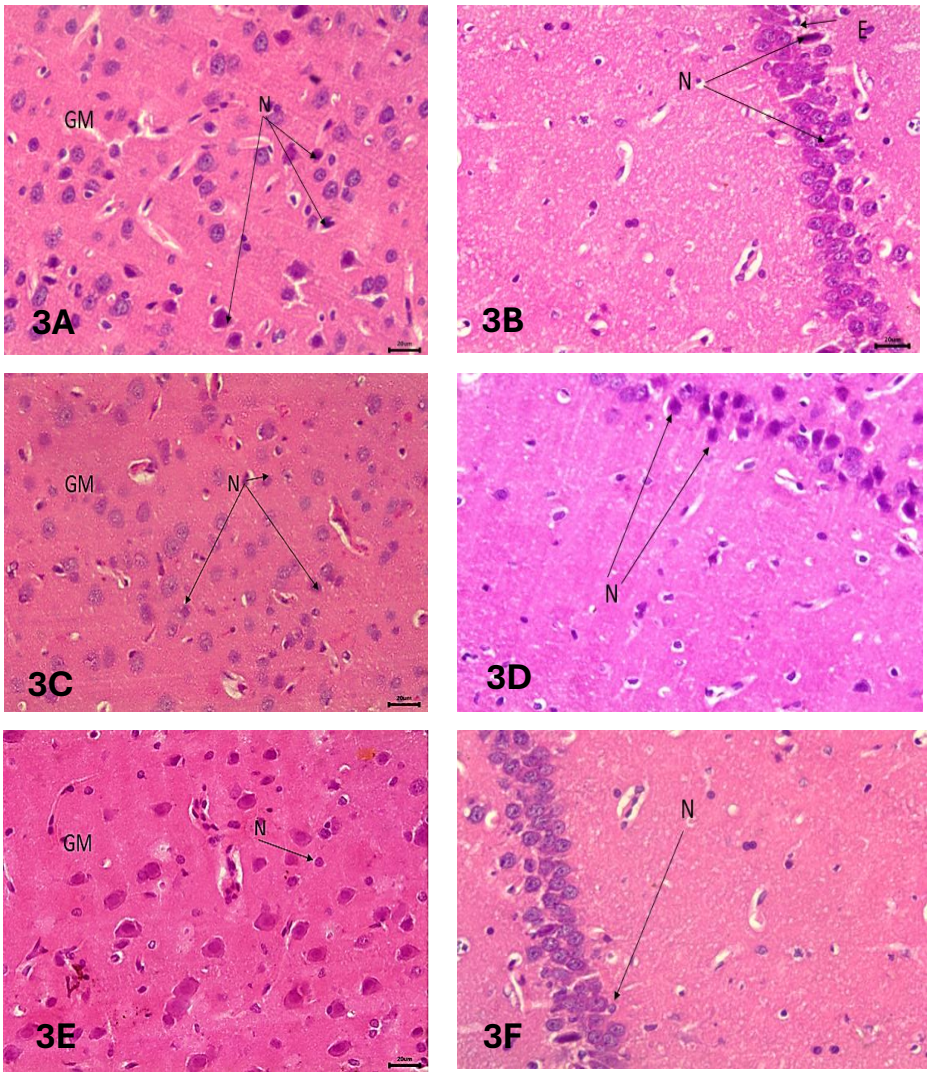

Plate 3 **A:** Photomicrograph of the cerebral cortex (GM) (GM) of rats from Ibuprofen with BP-treated group (G5) showing necrosis (N) of some nerve cells. **3B**: Photomicrograph of the hippocampus from group (G5) showing necrosis (N) of few nerve cells and perivascular edema. **3C**: Photomicrograph of the cerebral cortex (GM) from Ibuprofen with DPP-treated group (G6) showing necrosis (N) of some nerve cells. **3D**: Photomicrograph of the hippocampus from group (G6) showing necrosis (N) of few cells. **3E**: Photomicrograph of the cerebral cortex (GM) from BP and DPP along with Ibuprofen-treated group(G7), showing more or less normal nerve cells with few necrosis (N) of some nerve cells. **3F**: Photomicrograph of the hippocampus from group (G7) showing more or less normal structure with necrosis (N) of few nerve cells. H&E stain. scale bar =20µm.

The cerebellum of the control rats (G1) showed normal histological structure of the Purkinje cell layer (Plate 4A). The IBP-treated group (G2) showed demyelination in white matter and necrosis in the Purkinje cells (Plate 4 B), while the BP-treated group (G3) and DPP-treated group (G4) showed normal histological structure of Purkinje cells (Plate 4C; D). The BP+IBP group (G5) showed necrosis of a few nerve cells and Purkinje cells, and perivascular edema in their cerebellum (Plate 5A), with mild demyelination in white matter. The same pathological effects were detected in the cerebellum of the DPP-treated group (G6) (plate5 B). However, the cerebellum section of rats which were treated with combined BP and DPP along with IBP (G7) showed necrosis of a few Purkinje cells with mild demyelination in white matter (Plate 5C).

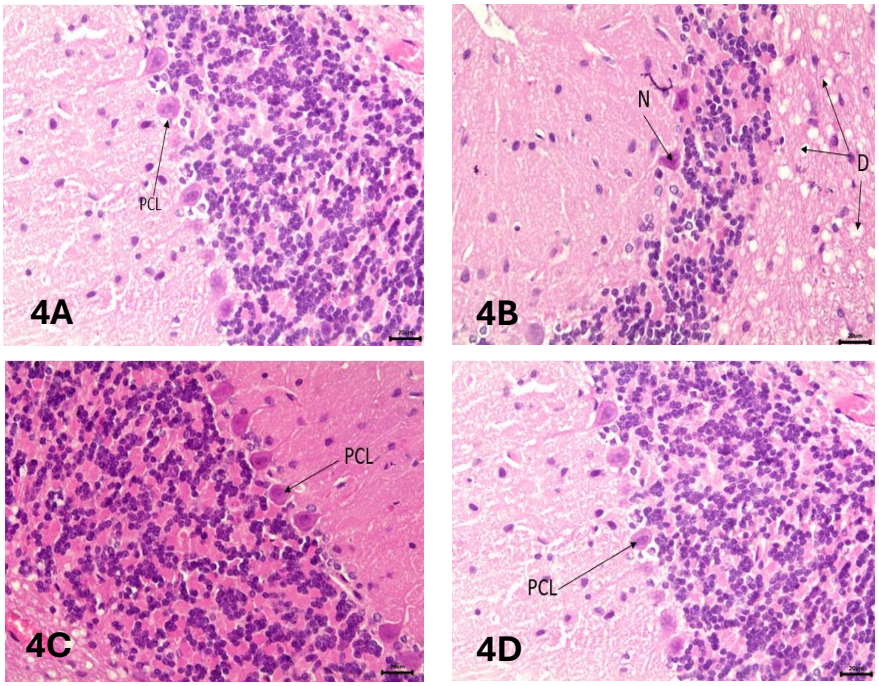

Plate 4 Photomicrograph of the rat cerebellum. 4**A:** The control group (G1) shows a normal histological structure of Purkinje cell layer (PCL). **4B**: The Ibuprofen-treated group (G2) shows demyelination (D) in the white matter and necrosis (N) in the Purkinje cells. **4C**: The BP-treated group (G3) shows a normal histological structure of Purkinje cell layer (PCL). **4D**: The DPP-treated group (G4) shows a normal histological structure of Purkinje cell layer (PCL). H&E stain. scale bar =20µm.

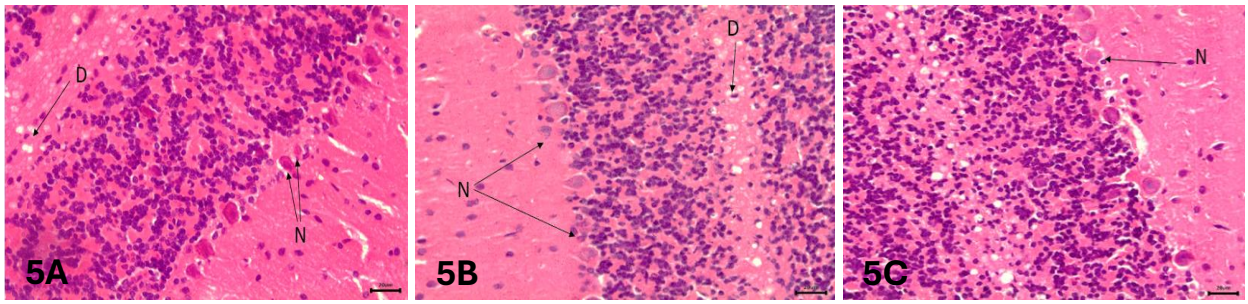

Plate 5A: Photomicrograph of rat cerebellum. The Ibuprofen + BP-treated group (G5) showing necrosis (N) of a few Purkinje cell layer mild demyelination (D) in white matter. **5B**: The Ibuprofen-treated group (G2) shows demyelination (D) in white matter and necrosis (N) in the Purkinje cell layer. **5C**: The BP, DPP and Ibuprofen-treated group (G7) showing necrosis (N) of a few Purkinje cell layer and mild demyelination (D) in white matter. H&E stain. scale bar =20µm.

DISCUSSION

IBP is harmful at the utilized concentration, according to pathological alterations in the brain's structure found in this study. Reactive oxygen species (ROS) production may be the cause of its toxicity. The brain's high metabolic activity and limited ability to regenerate compared to other tissues make it very vulnerable to reactive oxygen species (ROS) (Andersen, 2004). Consider the human brain, which uses roughly 20% of baseline oxygen for its operations despite its modest size. For the brain to maintain a low gradient of ions, it needs oxygen to make adenosine triphosphate (ATP), and for neurons to produce energy from glucose metabolism (Emerit *et al.*, 2004). Oxidative stress as a biochemical condition develops several types of reactive species such as ROS. The production of ROS as a result of xenobiotics induced pathological changes in the structure of the vital organs causing damage to these organs. Many drugs, such as IBP, induce oxidative stress, resulting in development of pathological changes in the structure of vital organs, such as the brain. However, biological systems have defense mechanisms against the generation of reactive species types. Lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) are examples of endogenous antioxidant enzymes that are part of the defense mechanism systems. It has been reported that increased antioxidant biomarkers such as LPO, SOD and CAT can be considered indicators for pathological changes induced by IBP in the brain structure (Chinwe *et al.,* 2020) in experimental animals. Because IBP interferes with membrane-dependent activities, the histopathological alterations observed in the brain in this study may be related to oxidative stress (Uzun *et al.,* 2010). These pathological changes in the brain structure in the IBP-treated group may potentially be caused by oxidative damage, which produces reactive oxygen species (ROS) that can harm proteins, lipids, and DNA, changing the integrity of the architectural membrane (Ozyurt *et al.,* 2004). The above suggestion was postulated, since very scantly information about the toxic effect of IBP on the brain structure. However, a study of El-sayed *et al.,* (2023a) illustrated that oral administration of sodium diclofenac (as NSAIDs) resulted in the histopathological changes in the brain structure of female albino rats. These changes accompanied with alteration in the antioxidant biomarkers. So, it can be concluded that the toxic effect of IBP on the brain structure may be derived from the increased oxidative stress induced by IBP.

Recently, natural herbal products have been used extensively to alleviate the toxic effects of drugs on the vital organs, despite the discovery of new synthetic drugs with antioxidant properties. The natural herbal product possesses powerful antioxidants due to the presence of phytochemicals such as phenolic acids, phenol amides and flavonoids in their compositions. These compounds were considered antioxidant, which eliminate the cellular damage via free radicals produced by synthetic drugs such as IBP. Ancient Chinese and Egyptians used natural herbal items called date palm pollen (DPP) and bee pollen (BP) for treatments of many diseases specially infertility in male and female humans (Hassan 2011; Nasser et al., 2020).

In the current research, oral administration of IBP Induced alterations in histopathology in the brain structure as demonstrated by necrosis of nerve cells with darkly stained nuclei, perivascular edema, increasing in the number of necrotic cells of hippocampus, and demyelination in white matter and necrosis in Purkinje cell layer in the cerebellum. However, administration of each. BP and DPP did not change the structure of brain (cerebrum, hippocampus and cerebellum) from that of the control group. But, administration of each BP and DPP along with IBP caused necrosis of some nerve cells of the cerebrum, and in Purkinje cell layer with mild demyelination in white matter of the cerebellum. On the other hand, the administration of combined BP with DPP along with IBP improved the structure, in spite of few necrosis of some nerve cells in the cerebrum and cerebellum being observed.

So, it can be concluded that administration of each BP and DPP along with Ibu not completely improved the brain structure. However, the combination of BP with DPP caused a marked improvement in the brain structure. These findings are in accordance with the study, which indicated that the administration of combined DPP with silymarin (as natural herbal antioxidant products) before sodium diclofenac (as NSAIDs) did not completely restore the brain structure, despite causing a marked improvement in this organ (EL-Sayed *et al.,* 2023).

The present study, to our best knowledge, bears originality regarding the existence of any previous studies in which BP and DPP were used in combination for the IBP-toxicity on the brain structure. In the future, the mechanism of BP and DPP (as antioxidants) for the potential role on the brain structure needs more studies.

CONCLUSION

From the results depicted in the present study, it can be concluded that the natural herbal products which possess antioxidant properties, such as BP and DPP or their combination, can be used in the protection of brain structure against IBP-induced histopathological alteration in this organ in male albino rats, Also, these natural antioxidant enzymes may strengthen the endogenous antioxidant enzymes, preventing the production of free radicals, such as ROS, causing damage in the vital organs, like brain.

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التأثير الوقائي العصبي لحبوب لقاح النحل ونخيل التمر ضد سمية الإيبوبروفين في ذكور الفئران البيضاء

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يتم إنتاج الأدوية المضادة للالتهابات غير الستيرويدية (NSAIDs) مثل الإيبوبروفين (IBP) عالميًا بكميات كبيرة لعلاج العديد من الأمراض. حبوب لقاح النحل (BP) هي أحد مضادات الأكسدة الطبيعية. يمكن استخدام منتجات النحل لعلاج الأمراض الالتهابية. حبوب لقاح نخيل التمر (DPP) المستخرجة من شجرة نخيل التمر تمثل الخلايا التناسلية الذكرية لزهرة النخيل وهى عنصر غذائي ممتاز، نظرًا لقيمته الغذائية وثرائه بالفينولات والفلافونويد مما يجعله مضادًا للأكسدة طبيعيًا. كان الغرض من هذه الدراسة هو التحقق من صحة التأثير الوقائي لمزيج حبوب لقاح النحل وحبوب لقاح نخيل التمر على سمية الدماغ الناجمة عن الإيبوبروفين. تم استخدام اثنين وأربعين (٤٢) من الفئران البيضاء لهذه التجربة. عولجت الحيوانات بـ حبوب لقاح النحل (١٠٠ مجم / كجم من وزن الجسم) و حبوب لقاح نخيل التمر (١٠٠ مجم / كجم من وزن الجسم) يوميا لمدة شهر واحد تليها الإيبوبروفين (٦٠ مجم / كجم من وزن الجسم) بشكل منفصل وأيضًا في تركيبة لمدة ثلاثين يومًا متتالية. كشفت هذه الدراسة عن تغييرات باثولوجية شديدة في بنية مناطق الدماغ الناجمة عن الإيبوبروفين .تم تحسين هذه التغييرات من خلال الجمع بين حبوب لقاح النحل و حبوب لقاح نخيل التمر. أظهرت هذه الدراسة أن حبوب لقاح النحل و حبوب لقاح نخيل التمر (كمضادات للأكسدة) لديها القدرة على الحماية من سمية الدماغ الناجمة عن الإيبوبروفين، وذلك بسبب قدرتها على استعادة بنية الدماغ وأنشطة الدفاع المضادة للأكسدة.