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Evaluation of *Tagetes Erecta* (Marigold) Extract (MaQxan™ (20:2)) potency in induced learning and memory deficit by modulating neurotransmission and synaptic plasticity

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The ancient Indian medical system, Ayurveda, has identified numerous plants with antioxidant properties as beneficial for treating neurological diseases. The active ingredients in herbal plants are utilized to modulate synaptic plasticity and neurodegenerative disorders. Different strategies are used to treat neuronal diseases and their secondary consequences by modifying synaptic plasticity. Because herbal remedies are inexpensive and have fewer adverse effects, they are advantageous. The main strategies for achieving this were to study the phases of *in vivo Tagetes Erecta* (Marigold) Extract (MaQxan^{TM} (20:2) administration in rats and, secondly, to study the potency of gene expression alteration on BDNF, TrkB, CREB, CAMKII, NMDA-R, nAChR, mGluR, and MAPK genes in the hippocampus. This study demonstrated that *Tagetes Erecta* (Marigold) Extract (MaQxan^{TM} (20:2) at medium and high doses (10 mg/kg to 20 mg/kg) modulated the genes for BDNF, TrKB, CAMKII, NMDA-R, nAChR, mGluR, and MAP-K, showing neurotransmitter and synaptic plasticity control efficacy in animal models. Our study advances knowledge of the effects of *Tagetes erecta* (Marigold) extract (MaQxan^{TM} (20:2)) on the central nervous system. Our results reveal that MaQxan^{TM} (20:2) significantly enhances memory retention and synaptic plasticity while maintaining neuronal integrity. Hence, the synaptic plasticity improvement shown by MaQxan^{TM} (20:2) can be attributed to the expression of plasticity-related proteins within the hippocampus, indicating its potential as a novel intervention for enhancing neurotransmission and cognitive function. These findings suggest that MaQxan^{TM} (20:2) may serve as an effective therapeutic strategy for improving synaptic plasticity and alleviating depression and stress-related cognitive deficits.

Keywords: MaQxan^{TM} (20:2), Neurotransmission, Sprague-Dawley (SD) male rats, Synaptic plasticity

The billions of nerve cells that make up the human brain connect with one another through these specialized synapses. A chemical neurotransmitter that binds to receptors on the second neuron is released from one neuron at each synapse. A signal is propagated when a neurotransmitter binds to the receptor of a second neuron, producing an action potential wave. Synapses are the fundamental units of brain communication. The majority of neuroscientists hold that the process of learning and memory formation involves reorganizing synaptic connections, eliminating certain synapses, and creating new ones. All synapses have the universal characteristic of synaptic plasticity, which is the capacity to modify synaptic strength in response to activity[1].

The relationship between stroke and cognitive damage is emphasized by PSCI, or post-stroke cognitive impairment. Therefore, developing focused intervention and treatment strategies requires an understanding of the intricate pathophysiological pathway linking stroke events to cognitive impairments. There is evidence that a stroke impairs synaptic plasticity, which could be a possible cause of PSCI (post-stroke cognitive impairment)[2]. The term "synaptic plasticity" describes how activity-dependent modifications to pre-existing synapses can alter the strength of synaptic connections and the effectiveness of synaptic transmission[3]. Synaptic plasticity can be classified into two categories: structural and functional. The process of structural synaptic plasticity describes how the number, density, and distribution of synapses can adapt to changes in the synaptic ultrastructure, emphasizing the strength of synaptic connections[4]. The efficiency of synaptic transmission, which includes long-term depression (LTD) and long-term potentiation (LTP), is often referred to as functional synaptic plasticity. Improving

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synaptic transmission efficiency and strength immediately improves information processing and storage in the central nervous system, which improves cognitive function[5]. These two factors are closely related to the recovery and improvement of cognitive impairment. While the precise processes underlying the improvement of cognitive impairment[6] have been demonstrated, techniques that regulate synaptic plasticity are still unknown.

Tagetes erecta L. is an annual herbaceous plant endemic to Mexico that is frequently referred to as "African marigold" or "Aztec marigold."This plant is widely grown for medicinal, poultry, and decorative purposes[7, 8]. Furthermore, since it is one of the most widely consumed edible flowers worldwide, its blossoms are utilized as a natural culinary color and as a component in salads[9]. The xanthophyll content and its application as an antioxidant to prevent age-related macular degeneration have been the main subjects of previous studies[10]. Phenolic compounds have received minimal study yet. It is crucial to build a comprehensive profile of composition and biological features because of these conditions as well as the wide range of uses. The marigold flower, or *Tagetes erecta* L., is an annual herbaceous plant that is widely cultivated for its bright yellow-orange flowers and used as a natural source of pigment. Numerous food products, such as cake mixtures, regular drinks, chewing gum, dairy analogs, egg products, fats and oils, processed fruit and fruit juices, and soups, use the flower's abundant carotenoids as a colouring agent[11,12]. Flowers from marigold plants are categorized as members of the Compositae family of medicinal plants. It is empirically used to treat fever, ulcers, burns, bronchitis, edema, and asthma[13]. The yellow-to-orange lutein pigments that make up the pigment content of marigold flowers are categorized as carotenoids[14]. Lutein for commercial usage is mostly derived from *T. erecta* L.

Health issues associated with aging and financial strain will rise as the number of elderly people increases. Consequently, the need for therapeutic approaches is essential. An infected brain is rare, whereas neurodegenerative disease is prevalent in older people. Human genes and environmental variables influence how neurodegenerative diseases progress. Parkinson’s disease (PD) and Alzheimer’s disease (AD) are the two neurodegenerative disorders that occur most frequently. Alzheimer's disease (AD) is a disorder that affects the elderly and results in memory loss and cognitive impairment because of neuronal degradation in the brain. It is characterized by gradual cognitive, behavioral, and memory deterioration (dementia), and it is linked to both mood swings and death. In the cerebral cortex, a buildup of intracellular neurofibrillary tangles and extracellular Aβ plaques is one of the pathophysiological mechanisms in AD[15].

According to the current literature review, Aβ-induced neurotoxicity, which results in Ca^{2+} dyshomeostasis, mitochondrial dysfunction, elevated oxidative stress, cholinergic dysfunction, and neuroinflammation, are the neurobiological mechanisms underlying AD[16]. These processes can lead to immune system malfunction, imbalanced lipid and cholesterol metabolism, deregulation of the protein degradation pathway, neuronal death, and neuronal/synaptic dysfunction. Studies conducted by others[17] also showed alterations in cortical and subcortical glutamatergic structures in AD. The nine biological hallmarks of aging that are linked to AD are stem cell exhaustion, telomere attrition, epigenetic alterations, proteostasis loss, mitochondrial dysfunction, genomic instability, and cellular senescence. Human degenerative diseases and aging are caused by an excessive production of reactive oxygen species (ROS), which oxidizes lipids, proteins, and DNA[18]. Previous research has demonstrated that AD is caused by cholinergic neuron loss, reduced acetylcholine, the absence of acetylcholine transferase indicators, and impaired choline absorption. The available alternatives for treating symptoms include NMDA receptor blockers and cholinesterase inhibitors[19]. AD treatment includes nerve growth factor stimulation, gamma aminobutyric acid receptor modulators, serotonin reuptake, somatostatin secretion stimulants, astrocyte modulating agents, phosphor-diesterase 4 inhibitors, and cannabinoid agonists[20]. Nevertheless, there are significant side effects associated with currently prescribed drugs, including bradycardia, low blood pressure, difficulty breathing, sleeplessness, nausea, lack of appetite, diarrhea, and muscle cramps and weakness[21, 22]. The amyloid-centric strategy for medication development for early disease prevention was the focus of certain investigations.

The present study investigated the impact of stroke pathology on synaptic plasticity, examined the alterations in synaptic plasticity associated with post-stroke cognitive impairment (PSCI), and outlined the potential benefits and underlying mechanisms of *T. erecta* (marigold) extract (MaQxan™ (20:2) in treating post-stroke cognitive impairment (PSCI) from

a synaptic plasticity perspective. MaQxan^{TM} (20:2) is a novel *T. erecta* (Marigold) extract formulation that combines the unique and individual effects of zeaxanthin and lutein in a synergistic manner, making it a completely novel drug that stands out from other products on the market.

Unlike other medications that only contain one of these essential components, this formulation contains lutein and zeaxanthin in a precisely balanced ratio, resulting in good absorption.

This study provided a good aspect for investigating the mechanism underlying post-stroke cognitive impairment (PSCI) and these results suggested that, the *T. erecta* (marigold) extract (MaQxan^{TM} (20:2)) can act as a potent source for synaptic plasticity and neurotransmitters.

Material and methods

Preparation of (MaQxan™ (20:2))

(MaQxan™ (20:2)) is manufactured and registered by Olive Lifesciences Pvt. Ltd., Nelamangala, Bangalore, Karnataka, India.

Experimental animals

In this study, male Sprague-Dawley (SD) rats aged 6 to 8 weeks were obtained from Chromed Biosciences in Bangalore, India. The guidelines for controlling and supervising experiments involving animals originated from CPCSEA Registration Number 1803/PO/RcBi/S/2015/CPCSEA. Rats were housed in autoclaved polypropylene cages with bedding made from autoclaved rice husk. There was a top grill on each cage, with room to store a water bottle and rodent food. Food and water that have undergone reverse osmosis (RO) were freely available to all of the animals. The animals had access to fresh, drinkable, and uncontaminated food and water. Periodically, the level of microbial contamination in food and water was determined. Drinking water bottles and the tubes that connected them were regularly inspected to ensure optimal operation. These cages were housed under standard laboratory conditions, with 22± 2°C and 44± 5% relative humidity, 12 hours of light and 12 hours of darkness. All animal-related procedures were performed in an ethical manner and under the direction of qualified experts. Before the study started, the research protocol was approved and assessed by Radiant Research Services Pvt. Ltd.'s Institutional Animal Ethical Committee (IAEC).

Dose administration

The experimental rats (Sprague-Dawley, SD) were randomly assigned to treatment groups and a control group (sedentary control, SC) after a week of acclimatization. The T2 (MaQxan^{TM} (20:2)) groups consist of three rats each and received different doses of 5 mg/kg, 10 mg/kg, and 20 mg/kg, respectively. These groups were categorized as high (T2H), medium (T2M), and low (T2L). The sedentary control groups (n = 3 rats) were pre-treated with a standard control Cytochrome c (Cytc) (Table 1).

Experimental design

Before receiving the medication injection, all animals were given one intraperitoneal (i.p.) anesthesia with a mixture of 70 mg/kg ketamine hydrochloride and 10 mg/kg xylazine. All medications were injected into the right hippocampal microspheres, which produced a reliable and efficient blockage of targets. Cytochrome c (Cytc) or antagonists were administered to sedentary rats. The injections were given to all rats once before the three days of running, early in the morning, to give them enough time to recover before they started running that evening. A wheel (31.8 cm in diameter and 10 cm in width) that could be freely revolved against a 100 g resistance was provided to the exercise rats, and it was observed on a regular basis. The rats in the control group were kept in a cage without a wheel. Wheel-running for three or seven nights was administered to the exercise rats. The right synaptic dissection was accomplished by visual inspection of the brain, and the placement of the microsphere injection was confirmed by histological analysis. For the purpose of isolating RNA, the sliced hippocampal tissue was stored at -80°C.

Isolation of total RNA from tissue samples and cDNA synthesis

The Invitrogen Pure Link TM RNA Mini kit was used to isolate total RNA from three treatment groups and one sedentary control group in accordance with the instructions provided by the manufacturer. Following the separation of RNA, quantification was

Table 1 — Differentiation of experimental groups

Groups	Sample name		Dosage
Group I	Control group	Sedentary control (SC)	Nil
Group II	(MaQxan^{TM} (20:2))	T2H	High (20 mg/kg)
Group III		T2M	Medium (10 mg/kg)
Group IV		T2L	Low (5 mg/kg)

done using 260 nm absorption. cDNA synthesis was performed using the extracted total RNA. Reverse transcriptase enzyme treatment was performed in accordance with the manufacturer's protocol (Bio-Rad) after cDNA was synthesized using oligo dT primers.

Analysis of mRNA expression by quantitative reverse transcription PCR (q-RT-PCR)

The CFX Opus 96 Real-Time PCR System (BIO RAD) detected the RT-PCR products directly without requiring further processing. That was used to measure the mRNAs for BDNF (Brain-derived neurotrophic factor), TrKB (Tropomyosin receptor kinase B), CREB (cAMP-response element binding protein), CAMKII (Calcium/calmodulin-dependent protein kinase II), NMDA-R (N-Methyl-D-aspartate), nAChR (Neuronal nicotinic acetylcholine receptors), mGluR (metabotropic glutamate receptors), and MAP-K (Mitogen-activated protein kinase), which were utilized for real-time quantitative reverse transcription polymerase reaction (RT-PCR). A dye-labeled DNA probe that was specific for the gene of interest and another that was specific for the gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were employed as an endogenous assay control. Then it was used to measure the increase in fluorescence to make this determination. 20 µL of the reaction mixture was amplified for gene expression using cDNA and primers that were specifically made for PCR. The number of RNA samples was standardized by using the endogenous control probe, which is unique to the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene.

RT-reaction steps (Amplification conditions)

After an initial incubation period of two minutes at 50°C, 30 minutes of reverse transcription at 600°C were conducted. The 40 cycles of two-step PCR reactions were considered to be 5 minutes at 95°C, 35 cycles of denaturation (30 seconds at 95°C), 30 seconds of annealing Tm (30 seconds at 72°C), and 45 seconds of extension at 72°C. Finally, for ten minutes, the temperature was raised to 72°C. Gene-specific forward and reverse primers were created using the NCBI primer blast algorithm for the BDNF (brain-derived neurotrophic factor), TrKB (tropomyosin receptor kinase B), CREB (cAMP-response element binding protein), CAMKII (calcium/calmodulin-dependent protein kinase II), NMDA-R (N-methyl-D-aspartate), nAChR (neuronal nicotinic acetylcholine receptors), mGluR (metabotropic glutamate receptors), and MAP-K (mitogen-activated protein kinase) genes in addition to GAPDH (glyceraldehyde-3-phosphate dehydrogenase). These primers were obtained from Eurofins, India (Table 2).

Statistical analysis

The RT-PCR result was analyzed by charting the intensity of the fluorescent signals on a semi-algorithmic scale versus the total number of PCR cycles. The amplification cycle at which the fluorescence increased significantly for the first time was called a threshold cycle (CT). The 2-ΔΔCT technique was used to determine the target genes' relative gene expression in relation to the reference gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase).

Results

The quantitative expression level of BDNF, TrKB, CREB, CAMKII, NMDA-R, nAChR, mGluR and MAP-K genes normalized to GAPDH in terms of percentage of expression. When compared with the sedentary control, high dose of MaQxan™ (20:2) (T2H, T2M and T2L) increase the mRNA expression of BDNF such as42.2, 59.3 and 10.9 % respectively in treated animal models (Table 3 and Figs 1-7).

Discussion

In an aging society, the enormous burden of AD is expected to increase in the ensuing decades. The importance of developing preventative measures to halt or reduce the disease in the preclinical or early stages of AD is highlighted by this realization (from

Table 2 — Primers used for Amplification

Sl. No	Gene	Primer Sequence (5′–′3)
1	BDNF	F-TGCAGGGGCATAGACAAAAGG
		R-CTTATGAATCGCCAGCCAATTCTC
2	TrKB	F-AAGGACTTTCATACGGAAGCTG
		R-TCGCCCTCCACACAGACAC
3	CREB	F-ACCATGGAATCTGGAGCCGAGAAC
		R-CTGTAGGAAGGCCTCCTTGAAAGA
4	CAMKII	F-ATTCAGCTCAGGATCTTTCGG
		R-AAGTCTGTCAGGCAGGCAGTCAGTT
5	NMDA-R	F-ACTCCACACTGCCCATGAAC
		R-TTGTTCCCCAAGAGTTTGCTT
6	nAChR	F-CCGTGTCACTGCTGAATCTGT
		R-CTCAAAGGACACCACGACAT
7	mGluR	F-TTTAGGTCAGAAGCCAGAGT
		R-CAGTAACCATCCTCTCTATCC
8	MAP-K	F-CGAAATGACCGGCTACGTGG
		R-CACTTCATCGTAGGTCAGGC
9	GAPDH	F-GTCTCCTCTGACTTCAACAGCG
		R-ACCACCCTGTTGCTGTAGCCAA

Table 3 — The quantitative expression level of BDNF, TrKB, CREB, CAMKII, NMDA-R, nAChR, mGluR and MAP-K genes normalized to GAPDH in terms of percentage of expression

Sl. No	Marker genes	Percentage mRNA expression in treated groups with different doses			
		MaQxan™ (20:2)			Sedentary control (SC)
		T2L	T2M	T2H	
1	BDNF	10.9	59.3	42.2	3.2
2	TrKB	9.8	30.6	27.7	5.9
3	CREB	0	0	0	0.09
4	CAMKII	5.8	32.3	38.4	0.5
5	NMDA-R	12.5	67.8	55.8	1.4
6	nAChR	31.2	35.6	44.4	8.8
7	mGluR	47.6	58.23	60.29	17.2
8	MAP-K	38.7	48.6	43.2	4.3

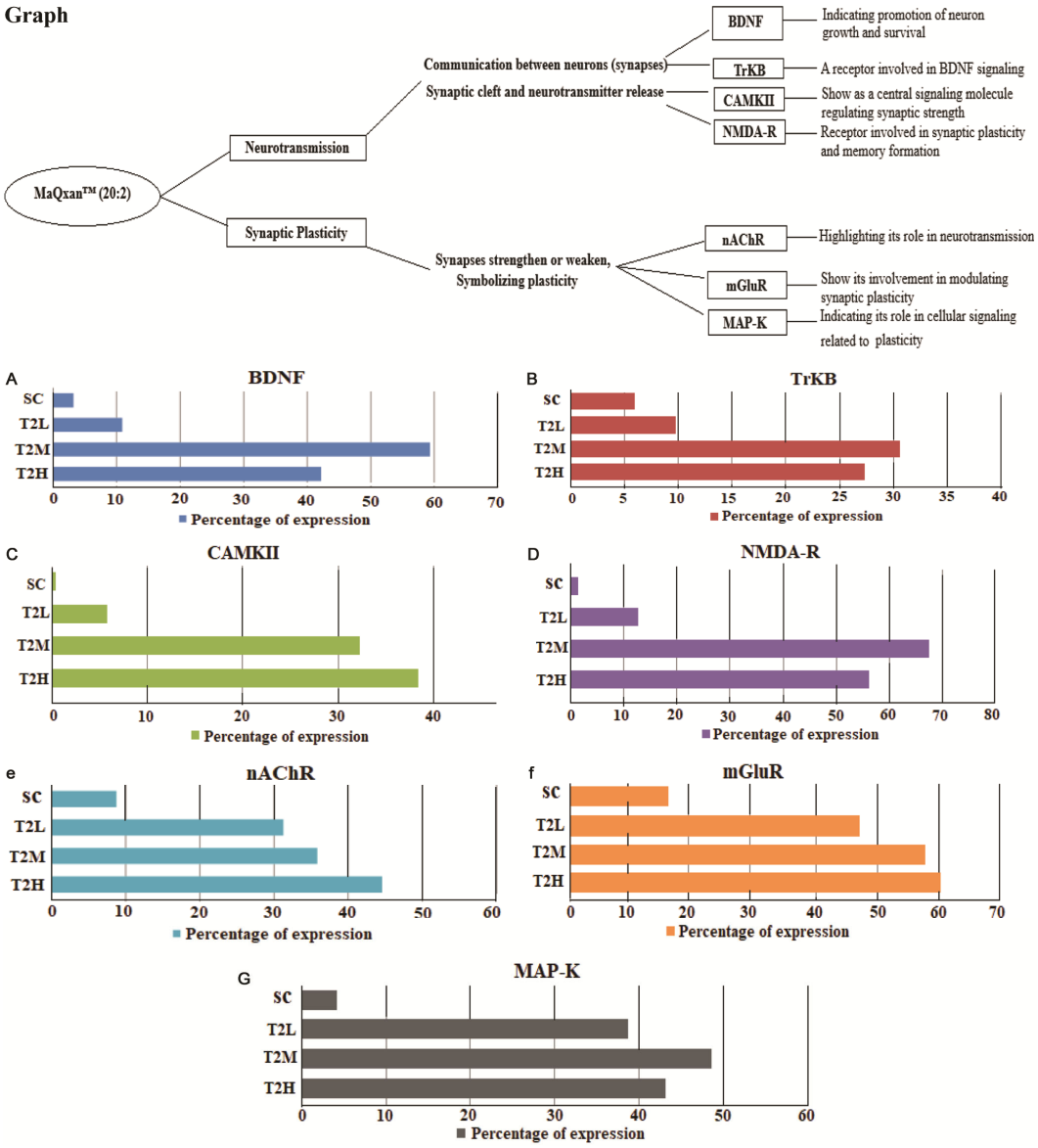

Fig. 1 — Percentage of (A) BDNF mRNA; (B) TrKB mRNA; (C) CAMKII mRNA; (D) NMDA-R mRNA; (E) nAChR mRNA; (F) mGluR mRNA; and (G) MAP-K mRNA expression induced by MaQxan™ (20:2) in different doses in treated rats and sedentary controls

mild cognitive impairment to mild dementia). One of the processes involved in the pathophysiology of AD is toxicity, which causes synaptic and neuronal degeneration in AD patients and transgenic rat models [23–26]. Even after decades of studies on dementia treatment, a thorough understanding of the pathogenesis and a viable treatment remain unattainable.

Therefore, the purpose of our study was to determine how MaQxan™ (20:2) modulates synaptic plasticity in the hippocampal region of the brain, which is primarily responsible for animal learning and memory. After administration of various dosages of MaQxan™ (20:2), the total RNA was extracted from the dissected hippocampal tissues of the treated animals. RT-PCR was used to determine the mRNA expression levels of eight marker genes, including BDNF, TrKB, CREB, CAMKII, NMDA-R, nAChR, mGluR, and MAP-K.

Brain-derived neurotrophic factor (BDNF), one of the eight flag genes, is a member of the neurotrophin family, which is essential for the development of the brain and spinal cord. In treated animal models, the BDNF gene boosted the ability of synapses to regulate synaptic plasticity and other cognitive functions 59.3% of the time, as well as the development of both excitatory and inhibitory synapses, and helped them mature. MaQxan™ (20:2) raised the mRNA expression of BDNF in treated animal models by 42.2, 59.3, and 10.9%, respectively, when compared to the sedentary control.

Tyrosine kinase B (TrKB) mRNA levels can be increased in tandem with BDNF, and this function serves as a loop to enhance BDNF's impact on synaptic plasticity. BDNF-TrkB signaling has been connected to multiple types of synaptic plasticity and was involved in transcription, translation, and protein trafficking during different stages of synaptic development. Mitogen-activated protein kinase (MAPK) is a gene involved in a series of signaling cascades that, when combined, perform many activities. In treated animal models, this study demonstrated that high and medium doses of MaQxan™ (20:2) increased the mRNA expression of TrKB and MAPK.

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Conclusion

One of the eight flag genes, brain-derived neurotrophic factor (BDNF), belongs to the neurotrophin family, which is essential to the development of the brain and spinal cord. The BDNF gene enhanced synapses' capacity to control synaptic plasticity and other cognitive processes in treated animal models 59.3% of the time. It also promoted the growth and maturation of both excitatory and inhibitory synapses. MaQxan™ (20:2) raised the mRNA expression of BDNF in treated animal models by 42.2, 59.3, and 10.9%, respectively, when compared to the sedentary control. Tyrosine kinase B (TrKB) mRNA levels can be increased in tandem with BDNF, and this function serves as a loop to enhance BDNF's impact on synaptic plasticity. BDNF-TrkB signaling has been connected to multiple types of synaptic plasticity and was involved in transcription, translation, and protein trafficking during different stages of synaptic development. Mitogen-activated protein kinase (MAPK) is a gene involved in a series of signaling cascades that, when combined, perform many activities. In treated animal models, this study demonstrated that high and medium doses of MaQxan™ (20:2) increased the mRNA expression of TrKB and MAPK.

In our study, MaQxan™ (20:2) is classified as a free lutein soft form rather than a powder, which may

influence its pharmacokinetics. Given that we conducted this preclinical research in animals, clinical studies involving humans may experience variations in administration, distribution, and elimination. A significant limitation of our investigation is the lack of available clinical efficacy data, which restricts our ability to draw definitive conclusions about the therapeutic potential of MaQxan™.

Conflict of interest

All authors declare no Conflict of interest.

Neurotransmission and Synaptic Plasticity Modulation by MaQxan™ (20:2)

MaQxan™ (20:2) (*Tagetes erecta* extract) modulates neurotransmission and synaptic plasticity by engaging a network of key signaling pathways and receptors. MaQxan™ (20:2) enhances neuro-transmission through nAChR (nicotinic acetylcholine receptors), facilitating synaptic communication, while promoting synaptic plasticity via BDNF (brain-derived neurotrophic factor) and its receptor TrkB (tropomyosin receptor kinase B). Activation of CAMKII (calcium/calmodulin-dependent protein kinase II) and CREB (cAMP response element-binding protein) further strengthens synaptic connections, contributing to long-term potentiation. Additionally, MaQxan™ (20:2) modulates NMDA-R (N-Methyl-D-Aspartate receptor), mGluR (metabotropic glutamate receptors), and MAPK (mitogen-activated protein kinase) signaling, which are crucial for memory formation and neuronal plasticity. These interconnected pathways suggest that MaQxan™ (20:2) promotes cognitive function through a multifaceted modulation of synaptic activity and neural adaptability.

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