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Kv1.3 blockade by PAP-1 decreases inflammation in LPS and IFNγ induced macrophages

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Abstract: Ethnobotany, the study of traditional plant use by indigenous cultures, provides valuable insights into medicinal applications that have stood the test of time. This review delves into the ethnobotanical practices surrounding plants used for brain health, juxtaposing traditional wisdom with modern scientific applications. Understanding brain health and addressing neurological conditions such as Alzheimer's, Parkinson's, and cognitive decline is of paramount importance. Traditional knowledge from various cultures, including Indigenous practices, Ayurvedic medicine, Traditional Chinese Medicine (TCM), African traditional medicine, and Native American practices, reveals a rich repository of plant-based remedies. Key plants like *Ginkgo biloba*, *Bacopa monnieri* (Brahmi), *Panax ginseng*, *Withania somnifera* (Ashwagandha), and *Huperzia serrata* have been traditionally used for their brain-enhancing properties. These plants contain active compounds such as flavonoids, alkaloids, terpenoids, glycosides, and saponins, which contribute to their neuroprotective, antioxidant, anti-inflammatory, and cognitive-enhancing effects. Modern pharmacology has begun to harness these ethnobotanical insights, integrating them into the development of standardized extracts and supplements. Clinical studies support the efficacy of these plants, but considerations of safety, proper dosage, and ethical sourcing are crucial. Despite these advances, challenges such as ethical use of traditional knowledge and conservation of ethnobotanical resources persist. The future of brain health treatments lies in the intersection of traditional practices and modern science, offering promising avenues for new discoveries and therapeutic applications. This review aims to highlight the importance of bridging these worlds, advocating for continued research and collaboration in the field of ethnobotany and brain health.

Keywords: Ethnobotany, brain health, neurological conditions, Alzheimer's, Parkinson's, *Ginkgo biloba*, *Bacopa monnieri* (Brahmi), *Panax ginseng*, *Withania somnifera* (Ashwagandha), *Huperzia serrata*, flavonoids, alkaloids, terpenoids, glycosides, saponins, neuroprotection, antioxidant, anti-inflammatory, cognitive-effect

Introduction: -Metabolic disorders such as obesity, type 2 diabetes, insulin resistance and non-alcoholic fatty liver disease have become major global health concerns due to their rapidly increasing prevalence and associated complications. In recent years, growing evidence has shown that chronic low-grade inflammation plays a central role in the development and progression of these disorders (Hotamisligil, 2006). Among the various immune cells involved in this inflammatory process, macrophages act as crucial player because of their ability to regulate both immune responses and metabolic functions. Macrophages are highly dynamic cells that can adapt their behaviour according to signals from their surrounding environment. Depending on these signals, macrophages can differentiate into either proinflammatory M1 macrophages or anti-inflammatory M2 macrophages. While M2 macrophages are generally involved in tissue repair and maintenance of metabolic balance, M1 macrophages are associated with inflammation and tissue damage (Odegaard & Chawla, 2011).

Under healthy conditions, adipose tissue contains a higher proportion of M2 macrophages, which help maintain insulin sensitivity and support normal metabolic activity. However, obesity and excessive nutrient intake disturb this balance and promote the recruitment and activation of M1 macrophages within metabolic tissues (Lumeng et al., 2007). Enlarged adipocytes release free fatty acids and inflammatory mediators that attract circulating monocytes into adipose tissue, where they differentiate into M1 macrophages (Weisberg et al., 2003). These activated macrophages produce large amounts of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), which contribute to a chronic inflammatory state. Persistent inflammation interferes with insulin signaling pathways and ultimately leads to insulin resistance and impaired glucose metabolism (Lauterbach & Wunderlich, 2017).

The role of M1 macrophages is not limited to adipose tissue alone. Increasing evidence suggests that these inflammatory cells also contribute to dysfunction in other organs involved in metabolic regulation. In the liver, M1 macrophages promote hepatic inflammation and fibrosis, accelerating the progression of non-alcoholic fatty liver disease. In pancreatic tissue, inflammatory mediators released by M1 macrophages impair β-cell function and reduce insulin secretion. Similarly, in blood vessels, these macrophages contribute to endothelial dysfunction and atherosclerotic plaque formation, thereby increasing the risk of cardiovascular diseases commonly associated with metabolic disorders (Odegaard & Chawla, 2011). Thus, M1 macrophage-mediated inflammation represents a critical link between obesity, metabolic imbalance and systemic complications.
Kv1.3 is a voltage-gated potassium channel involved in immune regulation and metabolic disease. It controls membrane potential and calcium signaling in macrophages, T cells, and adipocytes, thereby influencing inflammation and insulin sensitivity (Wulff et al., 2003). Increased Kv1.3 expression is associated with obesity, insulin resistance and type 2 diabetes due to enhanced pro-inflammatory cytokine production (Xu et al., 2004). In adipose tissue Kv1.3 promotes inflammatory activation that contributes to metabolic dysfunction (Upadhyay et al., 2013). Pharmacological inhibition of Kv1.3 improves glucose tolerance, reduces inflammation, and enhances energy expenditure, suggesting Kv1.3 as a promising therapeutic target for metabolic disorders. But scientific literature shows a mechanistic gap of role of Kv1.3 channel in M1 macrophages. PAP1 (5-(4-phenoxybutoxy)psoralen) is a potent, highly selective small-molecule blocker of the Kv1.3 voltage-gated potassium channel and is widely used in scientific studies (Schmitz et al., 2005).

The present study focuses on evaluating the effect PAP1, Kv1.3 inhibitor. PAP1 is reducing inflammatory markers associated with M1 macrophages.

Materials and Methods

Cell culture

Human derived monocytic cell line THP-1 was used for the study. The cells were maintained in RPMI 1640 media (Thermo) supplemented with 10% fetal bovine serum and 1% pen-strep antibiotic. For differentiation towards M1 macrophages: 500,000

cells were seeded in a six well plate and stimulated with 5ng/ml PMA (phorbol 12-myristate 13-acetate). After 24hours, cells were treated with 50ng/ml LPS (Lipopolysaccharides) and 20ng/ml IFNγ (Interferon gamma) with or without PAP1 (200nM) for 24hour. Cells were harvested after 24hour for further analysis. THP-1 cells were maintained similarly without any inducing agents and treated with PAP1 for 24 hrs qRT-PCR

500,000 cells were seeded and maintained as mentioned above. Post 24 hours of treatment, media was discarded, and 1ml of TRIzol (Takara Bio) reagent was added. RNA isolation was done manually, where 2μg of RNA was transcribed into cDNA using a cDNA synthesis kit (Thermo). Real-time PCR was performed using SYBR green (Bio-Rad). Beta actin was used as a housekeeping gene. Primer sequences used were as follows:

S.No	Gene	Forward Sequence (5'-3')	Reverse sequence(5'-3')
1	β actin	GACGACATGGAGAAAATCTG	ATGATCTGGGTCATCTTCTC
2	TNF-α	AGGCAGTCAGATCATCTTC	TTATCTCTCAGCTCCACG
3	IL-1β	CTAAACAGATGAAGTGCTCC	GGTCATTCTCCTGGAAGG
4	IL6	GCAGAAAAGGCAAAGAATC	CTACATTTGCCGAAGAGC
5	CD319	CATTGAAGAGAAGAAGAGAGTG	CTATTAGTGTGAGGGATTGTG
6	CD209	CGTAATCAAAAGTGCTGAGG	TAGATCTGAAAGTCCCATCC
7	CD36	AGCTTTCCAATGATTAGACG	GTTTCTACAAGCTCTGGTTC
8	PKM2	ATGTTGATATGGTGTTTGCG	ATTTCATCAAACCTCCGAAC
9	FAS	CTGTCCTCCAGGTGAAAG	TGTACTCCTTCCCTTCTTG

Data and Statistical Analysis

Relative gene expression was calculated using delta delta Ct method. All the experiments were done for at least three times. Student's T-test was used for calculating significance values where, *<0.05,**<0.01.

Result

PAP1 reduces inflammation in macrophages

Effect of Kv1.3 blocker pap-1 was assessed in THP-1 and its activated proinflammatory phenotype M1 cells. Transcript level of TNF-α was checked after PAP1 treatment. It showed phenotype specific response. TNF-α level was marginally increased in THP-1 whereas decreased in M1 cells upon PAP1 treatment (Fig 1 A & D). Earlier reports have shown reduction in TNF-α in known to have protection towards both inflammatory as well as metabolic conditions (Lin et al., 2008; Tesauro et al., 2008). Interleukin-1 beta (IL-1β) is a proinflammatory cytokine of the interleukin family which plays essential role in immune response, inflammation, cell communication among other functions. Kv1.3 blockade results in significant decrease in IL-1β gene expression in M1 phenotype while no change was observed in THP-1 cells (Fig 1 E & B respectively). This reduction is interesting because neutralization of IL-1β imparts reduction in disease severity in type 2 diabetes and rheumatoid arthritis (Dinarello, 2011). Next, IL-6 expression level was checked and it showed no significant change in both phenotypes after PAP1 treatment (Fig 1 C & F). Overall, PAP1 treatment shows significant reduction in proinflammatory markers in M1 cells.

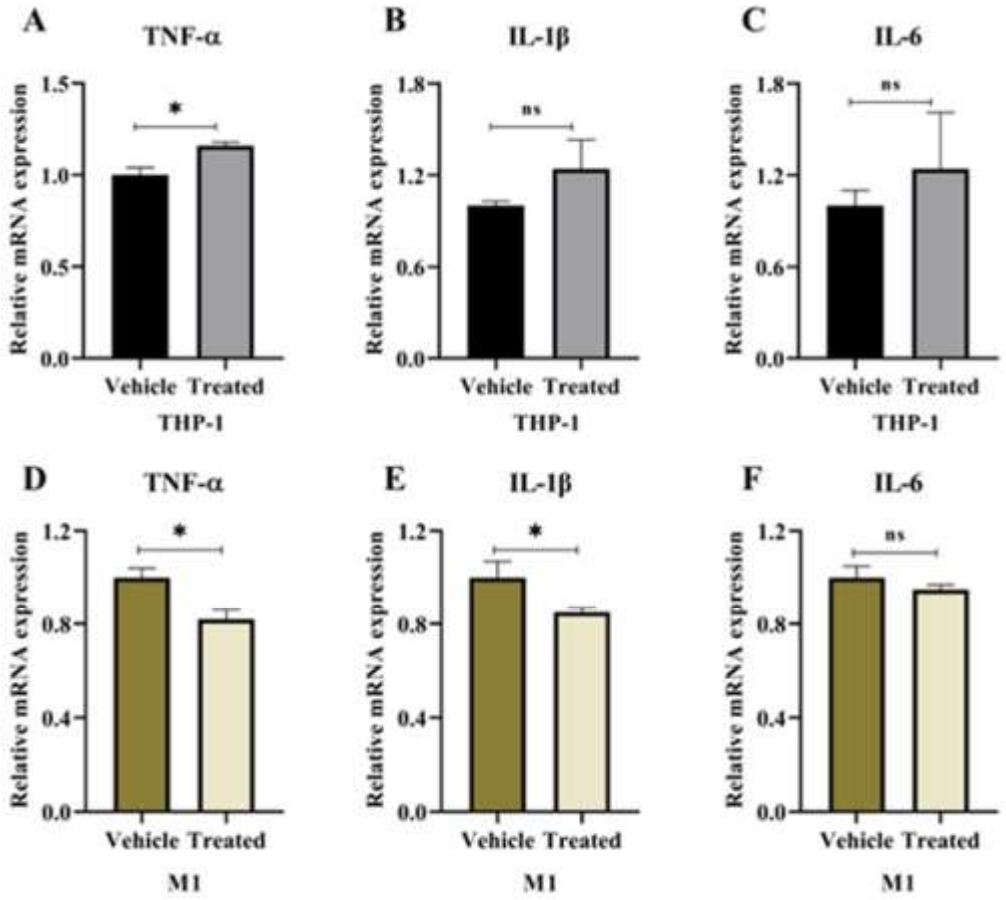

Figure 1: Effect of PAPI on inflammatory markers. TNF-$\boldsymbol{\alpha}$ in THP-1 and M1 (A &D), IL-1$\boldsymbol{\beta}$ in THP-1 and M1 (B & E) and IL-6 in THP-1 and M1 (C & F). Transcripts level checked using RTPCR. Data- Mean ±SD (n=3). *<0.05, ns= non-significant

PAP1 shows phenotype specific modulation of cell surface markers

Cell surface proteins are expressed on plasma membrane and helps in cellular responses like immune response and signalling in immune cells. CD319 is a cell surface protein which is upregulated in inflammatory macrophages (M1) and plays an important role in phagocytosis (Chen et al., 2017). CD319 expression level was checked after Kv1.3 blockade. Its expression was increased in THP-1 while no significant change was found in M1 cells (Fig 2 A & D). Increased CD319 in THP-1 cells shows enhanced immune response in THP-1 cells, this observation was also supported by enhanced TNF-α in THP-1 cells. Similarly, CD209 is a cell surface protein which helps in antigen presentation, cell repair processes and endocytic in nature are expressed in IL-4 treated macrophages (Tarique et al., 2015). PAP1 treatment showed no change in CD209 level in both macrophage phenotypes (Fig 2 B & E). CD36, a cell surface receptor also known as scavenger receptor belonging to class B member 3. They function in transportation of fatty acids and efferocytosis (Ferracini et al., 2013). PAP1 treatment shows significant increase in CD36 level in THP-1 while no change was observed in M1 cells (Fig 1 C&F). These results show PAP1 enhanced surface markers associated with proinflammatory pathways in THP-1 whereas, no significant change was observed in case of M1 cells.

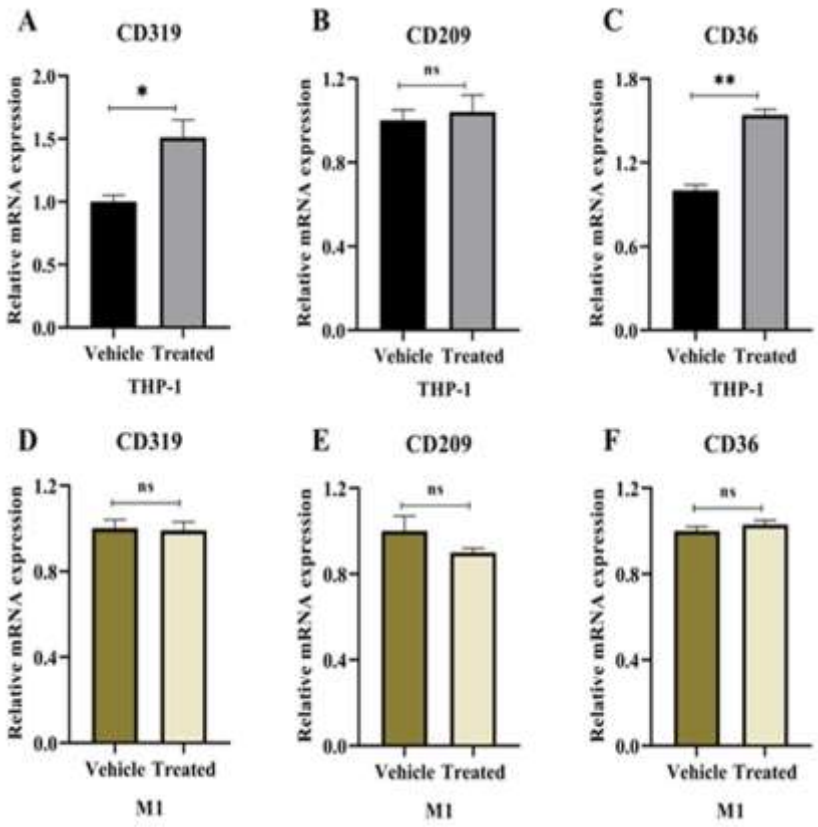

Figure 2: Effect of PAPI on surface markers. CD319 in THP-1 and M1(A & D), CD209 in THP -1 and M1 (B &E) and CD36 in THP-1 and M1(C&F). Transcripts level checked using RTPCR.Data- Mean±SD(n=3). *<0.05, ns=non-signifcant

PAP1 reduces the metabolic markers in inflammatory macrophages

Role of Kv1.3 channel in metabolic changes of macrophage phenotypes was assessed. PKM2 (Pyruvate kinase M2) is a glycolytic enzyme which fuels inflammatory activation (Patel et al., 2021). PAP1 treatment leads to increased PKM2 level in THP-1 while no change was observed in M1 cells (Fig 3 A&C). FAS (Fatty acid synthase) is a metabolic enzyme which catalyses formation of fatty acids and is linked with inflammatory conditions including cytokine production (Carroll et al., 2018). FAS has very interesting expression pattern after PAP1 treatment showing increased level in THP-1 while its level was subdued in M1 phenotype after Kv1.3 blockade (Fig 3 B&D).

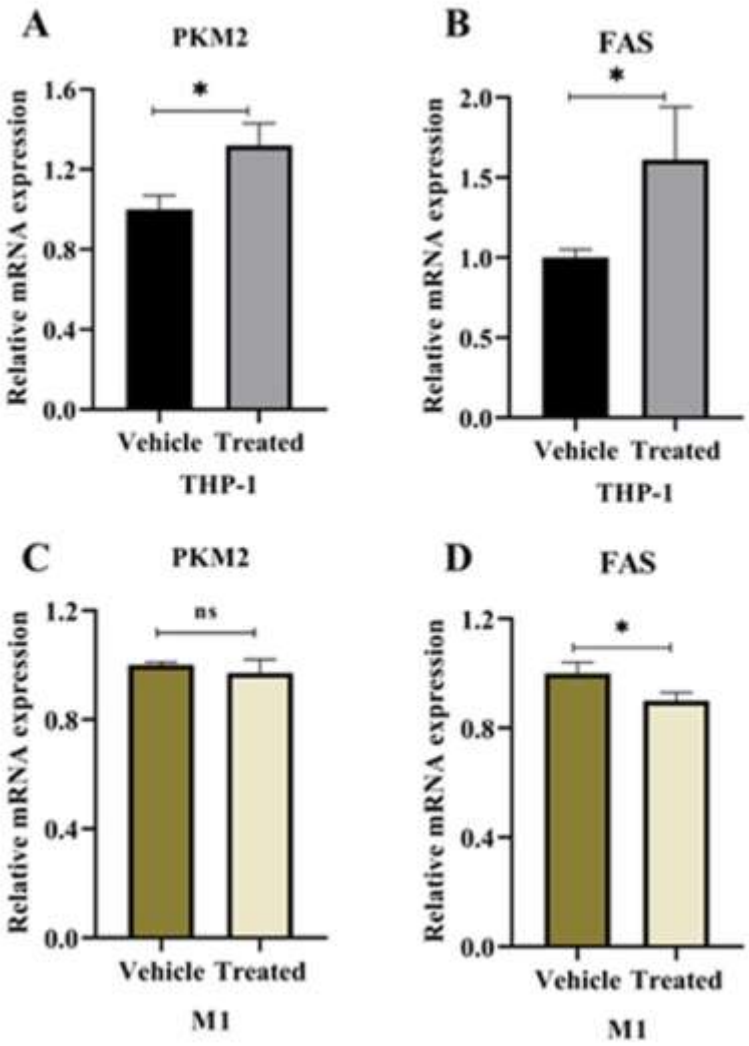

Figure 3: Effect of PAPI on metabolic markers. PKM2 in THP-1 and M1(A&C) and FAS in THP-1 and M1(B & D) Transcripts level checked using RTPCR. *<0.05, ns= non-significant

Discussion

The study highlights the role of Kv1.3 in macrophage function. The LPS and IFNγ induced macrophages are an established way to generate M1 macrophages in vitro. Our studies show that that blockade of Kv1.3 reduces inflammation. Earlier studies have shown the role of this channel in T cells activation (Chi et al., 2012). However, its role in macrophages is less studied.

This study is a direct outcome of our previous finding where Kv1.3 blockade in mouse model of obesity led to reduced TNF-α in adipose tissue and improved insulin resistance(Upadhyay et al., 2013). We argued that the adipose tissue has a larger population of macrophages compared to T cells, especially in obese conditions(Lumeng et al., 2007). Furthermore, it is known that during obesity M1 macrophage population increases significantly within adipose tissue. In the current study we find a direct evidence of ant-inflammatory effect of Kv1.3 blockade in M1 macrophages. It is also interesting to note that while TNF-α and IL-1β reduce upon PAP-1 treatment IL-6 does not change. IL-6, although classified as inflammatory cytokine, is known to improve insulin sensitivity (Carey et al., 2006).

The surface marker modulation brought about by PAP-1 is minimal and limited to M1 macrophages. It suggests that while THP-1 cells become more phagocytic upon treatment, there is no change in M1 macrophages. Similarly, the treatment seems to increase glycolysis (PKM2) and fatty acid synthesis in THP-1 cell. However, there is no change in glycolysis in M1 macrophages with decrease in fatty acid synthesis. In M1 macrophages FAS activity is directly related to prostaglandin synthesis and therefore, inflammation (D'Avila et al., 2011).

In summary, Kv1.3 blockade in macrophages reduces inflammation. This is not only evident from the

reduction in cytokines but also in activity of fatty acid synthase which is the main substrate for prostaglandin synthesis during inflammation. In context of obesity we now have a possible mechanism of how Kv1.3 blockade leads to reduction in adipose specific inflammation and an improved insulin sensitivity.

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