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Crosstalk Between miRNA-146a-5p and TRAF6/IRAK1/NF-κB Pathway in Adjuvant Induced Arthritis: Focus on Using Berberine as a Modulator of Inflammation

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Abstract

MicroRNAs have recently gained popularity because of their critical roles in the regulation of many biological processes. Their expressions are found to be attributed to different inflammatory disorders such as rheumatoid arthritis (RA). RA is a chronic autoimmune disorder that could be triggered due to genetic and epigenetic factors. Berberine (BBR) is an isoquinoline derivative alkaloid with a long history of medicinal use. It has wide anti-inflammatory, antibacterial, and antioxidative activities. However, the effect of BBR on miRNA-146a-5p (miR-146a-5p) expression level has not yet been studied. Thus, the present study is the first report that explores the exact mechanism of BBR on modulating miR-146a-5p expression in adjuvant induced arthritis (AIA) model. The arthritis severity scoring was assessed using hematoxylin-eosin and toluidine blue staining. Furthermore, immunohistochemical staining for the CD68+ macrophages expression was performed. MiR-146a-5p expression level was measured by reverse transcriptase quantitative real time polymerase chain reaction (qRT-PCR). The obtained results showed downregulation of miR-146a-5p associated with increased levels of antinuclear autoantibodies (ANA), tumor necrosis factor-α (TNF-α), IL-1β, nuclear factor kappa B (NF-κB), IL-6, interleukin-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6) and quantitative expression of CD68+ macrophages in AIA rat model. Remarkably, BBR treatment restores the levels of all these mediators. Collectively, the current results revealed the possible role of miR-146a-5p in the regulation of RA disease by manipulating TRAF6/IRAK1/NF-kB signaling as a macrophage inflammatory pathway. Furthermore, BBR showed potential ability to restore expression levels of miR-146a-5p and modulate NF-κB pathway proteins in AIA rat model.

Keywords: CD68+; Joint; Macrophages; Rheumatoid arthritis; Spleen.

Introduction

RA is an autoimmune systemic disorder described by the expansion of synoviocytes and other immune inflammatory cells as macrophages [1]. Macrophages are innate immune cells that produce growth factors, signalling molecules and cytokines thus orchestrating fundamental physiological processes inside the body [2]. Activated macrophages induce the progression of inflammation and aggravate joint destruction in RA [3]. Macrophage activation occurs *via* upregulation of NF-κB pathway [4] which regulates the transcription of inflammatory factors causing disease progression [5]. In inflammation, TRAF6 (an adaptor protein) is implicated in signal transduction [6]. It activates and interacts with other various protein kinases including IRAK1 [7]. The interaction between TRAF6 and IRAK1 leads to NF-κB pathway activation [8]. Recently, it is well believed that targeting the immune cells through altering miRNAs expression level presents an applicable therapeutic approach against multiple inflammatory diseases [9].

MiRNAs are a class of small non-coding RNAs of ~22 nucleotides that function as post-transcriptional controllers of gene expression [10]. Several reports indicated the role of miRNAs as potent biomarkers for diagnosis of many diseases [11]. MiR-146a belongs to miR-146 family and is found in intergenic region of chromosome 10q21 in rats [12]. MiR-146a plays a key role in immune regulation and prevents immune overreaction in several autoimmune disorders [13]. It seems to act

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through inhibition of NF-κB pathway *via* downregulation of its target genes, TRAF6 and IRAK1 [14]. Natural extracted compounds are a group of beneficial substances that are broadly used as antioxidant and anti-inflammatory agents [15]. Several plant extracted compounds were found to modulate miRNAs expression in cancer [16]. So, the targeting of gene networks and miRNAs using plant extracted bioactive molecules could be an effective strategy to combat RA development.

BBR *(*$C_{20}H_{18}NO_4$*+)* is an isoquinoline derivative alkaloid that is considered the most abundant constituent of *Hydrastis canadensis L*. It has been popularly utilized due to its impressive antibacterial, anti-tumour, and anti-inflammatory activities [17]. Several clinical and preclinical studies display an ameliorative effect of BBR against various conditions including metabolic, neurological and cardiological problems. It was found to suppress the immune responses and promote chondrocytes and osteocytes proliferation which ameliorate arthritis [18]. Importantly, many studies demonstrated the significant effect of BBR in the treatment of RA which qualifies it to be believed as one of the prominent outstanding natural products derived drugs in treatment of RA [19].

Since the developing of effective and safe therapy became an urgent need for RA treatment and owing to the tremendous regulatory role of miRNAs in the progression of different diseases, the present study aims to examine the role of miR-146a-5p in regulating TRAF6/IRAK1/NF-κB signalling as a macrophage inflammatory pathway in AIA through evaluating the prospective beneficial effect of BBR.

Material and Methods

Animals and ethics

All animal procedures were qualified by the Scientific Research Ethics Committee, Faculty of Science, Ain Shams University, with code: ASU-SCI/ ZOOL/2023/2/1 and were following the ARRIVE guidelines. 24 male albino Wistar rats (120-140 g) were obtained from Vacsera, Giza, Egypt. Rats were sheltered in the Animal House of Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University.

Treatment and dosage

Complete Freund's adjuvant (CFA) was purchased from Sigma Chemical Co. St. Louis, MO and used for arthritis induction as described previously [20]. Berberine chloride form (CAS Number: 633-65-8; Purity: ≥98% TLC) extracted from *H. canadensis* L. plant (the name was checked by https://wfoplantlist.org/) was purchased from Sigma Aldrich Chemical Co. USA. After 10 days of arthritis induction, BBR was administrated *via* intragastric route as 150 mg/kg body weight/day for 21 days [21].

Animal grouping

Basically, the 24 rats were assigned into four equal groups, the first group represented the normal control one. The second group was administered CFA to induce adjuvant arthritis (AIA). The third group was arthritic induced and then intragastric administrated BBR on day 10 for three weeks (AIA+BBR). The fourth group was administrated ~~with~~ BBR for three weeks (BBR) at the same dose and route as the third group. Animals' body weights were tracked to record weight gain throughout the experiment duration. Also, paw thickness in mm was measured using a caliber at day7, day14 and day21 after arthritis induction. At the end of experimentation, animals were sacrificed by an IV injection of 1.25 mg/Kg of sodium barbital, and plasma samples were used for measurement of miR-146a gene expression. Moreover, dissected spleens were cleaned with saline and homogenized for the evaluation of different immunological parameters. Samples of the left hind paw were immersed separately in neutral buffered formalin for histological and immunohistochemical investigation.

Assessment of arthritis severity

Joint sections were cut and stained with H&E to evaluate the degree of leukocyte infiltration, synovial hyperplasia, and bone erosion. Also, toluidine blue stain was used to assess cartilage damage through proteoglycans microscopic evaluation. Histologic scores were reported according to the infiltration of inflammatory cells, synovial hyperplasia, bone erosion, and cartilage destruction. The scoring criteria was monitored as formerly described [22].

Detection of ANA and different immunological parameters

ANA level was carried out by enzyme linked immunosorbent assay (ELISA) in spleen tissue homogenates using ELISA kit with catalogue number (cat. no.): MBS269217. Evaluation of spleen tissue levels of the inflammatory mediators was carried out by quantitative Rat ELISA Kit according to the manufacturer's directions as follows: IL-1β, IL-6 and TNF-α by Quantikine ELISA rat kit; Kamiya Biomedical Company; cat. no.: KT-18885, KT-19418 and KT-30800 respectively. NF-κB by rat Nuclear Factor Kappa B ELISA kit; CUSABIO Company; cat. no.: CSB-E13148r. TRAF6 by rat TNF receptor associated factor 6 ELISA kit; Genie Company; cat. no.: RTEB1603. IRAK1 by rat interleukin 1 receptor associated kinase ELISA kit; LifeSpan BioSciences Company; cat. no.: LS-F17880.

Immunohistochemical staining for CD68+ cells expression

Immunohistochemistry was achieved on joint tissue sections from all experimental animals for detection of CD68+ cells. The sections were treated

with 10 mL Mol Tris buffer and 1 mL Mol ethylenediaminetetraacetic acid. The sections were submerged in 3% H_2O_2 to inhibit the endogenous peroxidase, and then incubated in 1% bovine serum albumin at 4°C [23]. The sections were stained using rabbit polyclonal anti-CD68 (Abcam, Cambridge, UK) for demonstration of macrophages. For quantitative analysis of the immunohistochemical staining, Tin Eye labs_color Extraction Lab= software was accomplished.

Analyzing of miR-146a-5p targets by miRSel database

MiRNA-146a-5p target genes were analysed through miRSel bioinformatics database that connects text-mining outcomes to the existent databases and computational predictions [24].

Establishment of gene network maps for the obtained genes Il1b, Irak1, and Traf6

The attained target genes of miRNA-146a-5p were investigated for construction of their gene network maps through use of GeneMANIA bioinformatics database V. 3.6.0 according to Warde-Farley *et al.* [25].

Measurement of miR-146a-5p expression levels by qRT-PCR analysis

Total RNA was extracted from plasma according to instructions provided by the manufacturer using RNA isolation Kits purchased from Favorgen Biotech (Taiwan), cat. no. FABRK001. After extraction, one-step qRT-PCR kit (SYBR Green, low Rox) purchased from Enzynomics, South Korea, was utilized for cDNA synthesis. Also, specific primer pairs were designed using miRprimer software [26]. The miR-146a-5p relative expression were normalized against U6 as a universal endogenous reference gene (Table 1). The relative expression of rno-miR-146a-5p was determined according to $2^{-\Delta\Delta CT}$ low [27]. while its mature sequence was reclaimed from miRBase database (Accession number: MIMAT0000852) [28].

Statistical analysis

The Shapiro-Wilk test was used for normality distribution analysis. Mean ± S.E. and percentage of change from control were assessed. Statistical difference between groups was recorded by one-way analysis of variance (ANOVA) test and LSD multiple comparisons test in which $P<0.05$ is expressed significant.

Results

Effect of BBR on paw inflammation in AIA

At the beginning of the study, no marks of inflammation were noticed in rats' paws in any experimental group. Following arthritis induction, marks of swelling and redness were noted in the injected paws as illustrated in Figure1 that were extremely perceived in AIA group. However, inflammatory symptoms were ameliorated in BBR treated animals as paw swelling thickness decreased from 0.60± 0.05 in AIA group to 0.44± 0.05 in AIA+BBR group.

Effect of BBR on Body weight gain in AIA

Tracking weight gains in animals of the normal control group showed gradual increment in means from 159.83±6.25 reaching to 212.00±5.18 at the end of the experiment producing a percent of increase by 32.6%. On the contrary, AIA rats expressed weight loss reaching a mean body weight of 131.83±2.82 at the end of the study with a reduction percent of 12.30% which was ameliorated to a percent of increase by 23.99% in AIA+BBR rats (Figure 2).

Effect of BBR on arthritis severity scoring in AIA

As shown in Fig. 3, articular tissue of AIA group was represented by inflammatory cell infiltration (Fig.3A), synovial hyperplasia (Fig.3C), and bone destruction (Fig.3E) as shown in H&E-stained slides. Moreover, cartilage damage was demonstrated *via* toluidine blue stain (Fig.3G). These histological features displayed less severe features in AIA+BBR animals (Fig.3B, D, F, H) in relation to AIA animals. To quantitate these changes, the parameters were scored as a measurement of arthritis severity in the treated group compared to AIA rats. In BBR treated rats there was a significant reduction ($p<0.05$) in arthritis severity scoring indicators compared to AIA rats (Fig.3I).

Effect of BBR on spleen ANA levels in AIA

AIA rats recorded a significant rise *($P<0.05$)* in ANA level compared to normal control. However, treatment with BBR for three weeks caused a significant decrement in ANA levels compared to AIA rats (Figure 4).

Effect of BBR on macrophage inflammatory mediators' spleen levels in AIA

Currently, Figure 5 presents spleen tissue content of IL-1β, IL-6 and TNF-α as the main inflammatory mediators secreted from macrophages in adjuvant arthritis. Results of this study recorded a significant increment in IL-1β, IL-6 and TNF-α in AIA group when compared to their controls. Conversely, statistically significant amended results *($P<0.05$)* in these levels were obtained in AIA+BBR treated animals compared to AIA group. TRAF6, IRAK1 and NF-κB were also demonstrated in Figure 5 representing one of the main transcription signaling pathways of macrophages. It was revealed that there is a significant escalation in TRAF6, IRAK1, and NF-κB spleen levels in AIA group recording 593.37±4.79, 537.13±1.65 and 12.21±0.04 respectively compared to controls. While BBR treatment significantly decreased TRAF6, IRAK1, and NF-κB spleen levels in AIA+BBR group

recording 518.37±1.65, 314.3±0.81 and 8.07±0.02 respectively compared to AIA group.

Effect of BBR on expression levels of CD68+ reactant macrophages

Regarding immunohistochemical staining, CD68+ reactant cells expression displayed negative reaction sites in joint sections from control and BBR groups (Fig. 6A&D respectively). But excessively reactant sites for CD68+ were observed in joint sections from AIA group showing strong CD68+ immunoreactivity in macrophages (Fig. 6B). On the other side, joint section from AIA+BBR rats (Fig. 6C) showed decreased CD68+ macrophages compared to AIA group. Also, quantitative analysis of CD68+ reactivity was demonstrated in Fig. 6E.

Effect of BBR on miR-146a-5p expression levels

In real-time PCR analysis of miR-146a-5p, data illustrated in Figure 7 represented down-regulation in mature rno-miRNA-146a-5p in AIA group relative to the normal control. Worthy of being noted, following BBR administration, a significant elevation was recorded in rno-miRNA-146a-5p expression in AIA+BBR group when compared to AIA group.

Analyzing of miR-146a-5p targets by miRSel database

By applying search criteria on the miRSel database engine, it showed 9 target genes associated with rat miR-146a expression which included IL1b, Traf6, and Irak1 encoding for IL-1β, TRAF6, and IRAK1 inflammatory mediators under investigation (Table 2).

Establishment of gene network maps for the obtained genes Il1b, Irak1, and Traf6 by GeneMANIA database

By GeneMANIA bioinformatics tool, Fig. 8 shows the genes network maps of Il1b, Traf6, and Irak1 genes, to establish the possible correlations in gene partners, and predict their functions and pathways. The data suggested that TRAF6 interacts with 20 targets and could regulate interleukin 6 production and I-kabbaB kinase/NF-kabbaB signaling in addition to its effect in cellular response to interlukin-1. Moreover, Irak1 and IL1b each interact with 20 targets and could regulate several processes including cytokine action, cytokine mediated signaling pathway, cytokine receptor binding, response to heat and regulation of interleukin 6 production (Fig. 8).

Discussion

The role of miRNA-146a in autoimmune diseases has become a popular research topic specially in RA [29]. Currently, paw swelling, body weight gain, and ANA were considered as encouraging signs for arthritis. AIA animals showed a significant decline in body weight compared to their initial body weights accompanied with signs of inflammation in their paw's joints as well as a significant increase in ANA levels which all authenticate arthritis development in rats. These results were in accordance with Abo-Aziza *et al.*, [20]. On the contrary, decreased paw swelling, increased zest for food and significant decrease in ANA levels were attained after BBR treatment. This improvement is due to the role of BBR in lowering inflammatory markers [30]. These results were interrelated to the significant reduction in arthritis severity scoring in AIA+BBR group in comparison with AIA group. Huang *et al.*, [19] verified that the anti-inflammatory role of BBR occurs through inhibiting proliferation of inflammatory immune cells. Moreover, BBR treatment can reinstate the balance of the macrophage1/macrophage2 by lowering the levels of M1 proinflammatory cytokines and increasing the levels of M2 anti-inflammatory cytokines [31]. Macrophages are one of the highest frequent cell types in the synovium of RA, thus developing new therapies to target them became of great interest in RA treatment [3].

Presently, AIA rats illustrated a significant elevated spleen levels in IL-1β, IL-6, TNF-α when compared to the normal control animals. These results were in agreement with Abo-Aziza *et al.*, [20]. This may be due to activation of macrophages which was hereby mirrored as a significant elevation in quantitative expression of CD68+ macrophages in immunohistochemical staining joint sections of AIA group compared to controls. Similarly, H&E joint sections of the current results visualized a heavy inflammatory cells infiltration of AIA group. Also, it was proved that the number of infiltrating macrophages in the synovial tissue were associated with disease activity and bone erosion [3]. Concerning macrophage cytokines, IL-6 can stimulate osteoclasts leading to pannus development. Both IL-6 and IL-1β elevate matrix metalloproteinase (MMPs) secretion, leading to cartilage damage [3]. IL-1β causes an overexpression in endothelial cell adhesion molecules, that is crucial in macrophages migration to the inflamed tissue [32]. TNF-α stimulates the synthesis of collagenases in articular cartilage, causing the activation of osteoclasts and, giving rise to bone erosion [33]. However, in the current results, these proinflammatory mediators were significantly reduced in AIA+BBR group compared to AIA group. This was accompanied with a significant decline in quantitative expression of CD68+ macrophages in immunohistochemical staining joint sections which was in line with previous results [34]. This modulatory effect of BBR occurs through suppression of proinflammatory reactions through adenosine 5'-monophosphate-activated protein kinase (AMPK) stimulation in macrophages that decreased TNF-α, IL-1β, and IL-6 production [19]. So, herbal medicines containing BBR are widely

used in RA treatment and recently BBR was considered as one of the most encouraging natural products in RA treatment.

Undoubtedly, it is well known that activation of macrophages occurs *via* upregulation of NF-κB pathway [4]. However, the interaction between TRAF6 and IRAK1 leads to activation of transforming growth factor-β activated kinase 1, which in sequence triggers NF-κB pathway [8]. A significant elevation in spleen levels of NF-κB, TRAF6 and IRAK1 in AIA group compared to control group were noted. These data are in accordance with former experiments that identified the role of TRAF6 in macrophages [35, 36]. It was demonstrated that inhibition of TRAF6 hinders macrophage inflammation [37]. The upregulation in TRAF6 expression in RA directly correlates to synovial tissue severity and inflammatory cells infiltration [38]. Moreover, it was exhibited that TRAF6 promotes M1 polarization to aggravate neuropathic pain in chronic constriction injury mice model [37]. Additionally, IRAK1 is also related with the vulnerability to autoimmune diseases and might be one of the major risk factors of RA [35]. IRAK1-deficient macrophages exhibit a reduced activation of NF-κB pathways [39]. In the present work, spleen levels of NF-κB, TRAF6 and IRAK1 were significantly reduced in AIA+BBR group compared to AIA group. These results are highlighting the ability of BBR in inhibiting the activation of NF-κB pathway [21, 40, 41]. Nonetheless, these decreased levels after BBR administration might be due to the ability of BBR to increase the levels of IkB proteins which inhibit the activation of NF-kB [42]. However, it was illustrated that the immunomodulatory effect of BBR is based on adapting multiple signalling pathways including AMPK/NF-κB, leading to the inhibition of inflammatory signals that are responsible for joint inflammation and bone erosion [18]. miR-146a is a well-popular negative feedback mediator of inflammatory response since it engages in the regulation of the development of various inflammation-related diseases including RA [43]. It acts as a principal role in the control of innate immunity and its relation to adaptive immunity [44].

The current research has examined the role of miR-146a-5p in regulating TRAF6/IRAK1/NF-κB signalling as a macrophage inflammatory pathway in AIA through the effect of BBR. It is worth mentioning that, up to date, there are no published articles discussing or testing the role of BBR in enhancing the expression of miR-146a in AIA model or even in RA, making the current study of noticeable interest. Currently, a significant downregulation in the expression level of miRNA-146a was obtained in animals of AIA group compared to the controls which was in harmony with Zhao *et al.*, [41]. However, Zhao *et al.*, [45] observed that a reduction in the expression level of miR-146a was associated with joint inflammation in RA patients. Moreover, a diminution in miR-146a expression level in osteoarthritic patients with increasing cartilage degeneration was demonstrated [46]. In another aspect, King *et al.*, [47] reported that miR-146a deficient mice consequently developed chronic inflammation and autoimmune diseases. These present findings can be explained through the correlation between miR-146a and its target genes. Currently, by using miRSel database, rno-miRNA-146a was found to regulate nine target genes including IL1b, Traf6, and Irak1 genes that encode for IL-1β, TRAF6, and IRAK1 respectively. Moreover, the elevated levels of these 3 inflammatory mediators in AIA group verified clearly the obtained miRSel outcomes that recoded a significant downregulation in miR-146a expression level in such group compared to controls. Thereafter, the present work subjected these 3 target genes to further analysis by GeneMANIA to construct for gene networks and functions as well. It was mentioned that miR-146a suppresses NF-κB signalling and inhibits the production of the inflammatory mediators [48] explaining the elevated level of macrophages cytokines in AIA animals. In the same manner, regarding TRAF6 and IRAK1, there is a negative feedback mechanism regulating the correlation between miR-146a and inflammatory pathways as TRAF6 and IRAK1 [49] thus also effecting NF-κB pathway. miR-146a is considered a NF-κB dependent gene that is normally capable of inhibiting IRAK1 and TRAF6 expression via binding to their mRNAs' 3'UTR [8, 43]. In RA, it was shown that miR-146a can improve disease progression via complementing and binding with the 3′- UTR of TRAF6, IRAK1 and NF-κB mRNA, inhibiting their expression and deteriorating the downstream signal transduction [50, 51]. Also, it was confirmed that the deficiency of miR-146a-5p in macrophages enhances inflammation through increasing the TRAF6-IRAK1 complex and NF-κB signalling pathways [52]. Another explanation for the obtained decrease in miR-146a expression level in AIA could be due to the activation of NF-κB signalling that leads to an increment of IL-1 levels that activates MMPs [53] which causes a decrement in the expression of miR-146a [46]. On the other hand, and interestingly, the present results revealed a remarkable upregulation in miRNA-146a expression level in AIA+BBR animals in comparison with AIA animals. This current upregulation in miR-146a is perhaps due to the role of BBR as putative target to miRNAs [54]. BBR applies anti-inflammatory effects by the regulation of several types of miRNAs in different diseases. In this line, Li *et al.*, [55] discovered the potential role of BBR to promote miR-145 expression in human ovarian cancer. Likewise, Ren *et al.*, [56] emphasized a significant overexpression of miRNA-188-5p levels in melanoma treated with BBR. Also, treatment with BBR inhibits NF-κB nuclear

translocation via reducing levels of miR21 in multiple myeloma cells [57]. BBR plays a therapeutic role by up-regulating the expression of miR-103a-3p and repairing the intestinal mucosal barrier in ulcerative colitis [58].

Overall, miRNAs regulation seems to possess molecular mechanism by which pharmacological activities of BBR are performed. More importantly, the present findings are most likely attributed to the major role of BBR which exerts antioxidative and anti-inflammatory activities that, in turn, inhibits the NF-κB, IRAK1 and TRAF6 levels, and eventually, leads to elevation in the miR-146a level through attenuation of the inflammatory response by negative feedback loop.

Conclusion

It is worth mentioning, that up to date, there are no published articles discussing the role of BBR in enhancing the expression of miR-146a in AIA, making the current study of noticeable interest. In this study, BBR therapeutic action seems to be *related* to the regulation of miR-146a-5p which can orchestrate TRAF6/IRAK1/NF-κB, the main inflammatory pathway in macrophage. As a result, BBR has the capability to be used as an effective therapy to inhibit the development of RA.

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Declaration of Conflict of Interest

The authors declare that they have no competing interests.

Ethical of approval

The protocol and all animal procedures were reviewed and accredited by the Scientific Research Ethics Committee–Faculty of Science–Ain Shams University, with code: ASU-SCI/ ZOOL/2023/2/1.

Availability of data and materials

Data and materials analysed during this study are mostly included in this article (additional raw data analysed in the current study are available from the corresponding author on reasonable request).

Authors' contributions

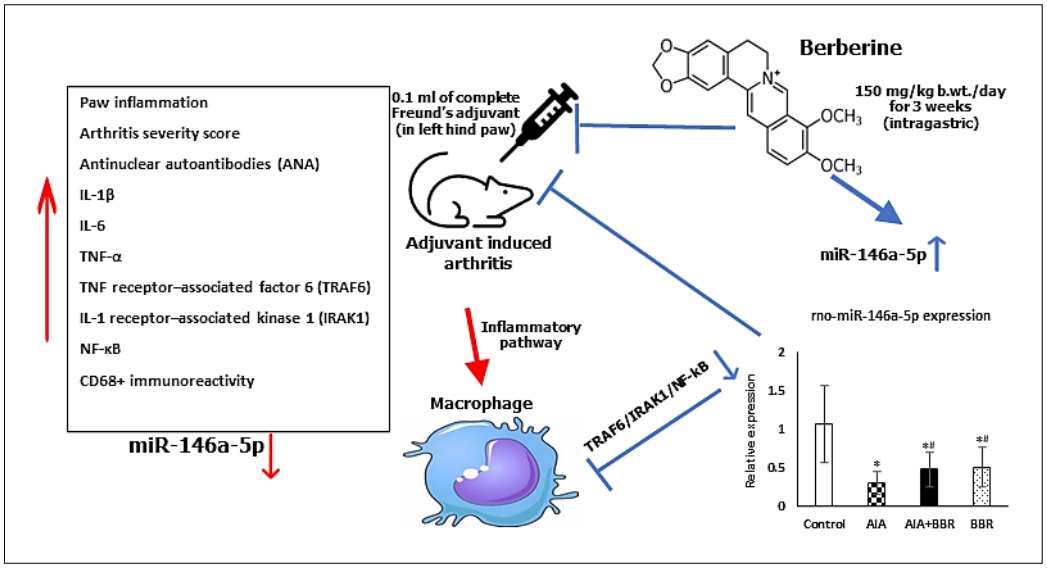
Main idea, experimental and statistical analyses, manuscript writing, and revision were performed by all authors.

TABLE 1. Primers sequences used in the one-step qRT-PCR

Primer	5'--3' primer sequence
rno-miR-146a-5p (forward)	**5'**-GGACGGTAGCAAGCAAAGAGTGTGAACCCATGGAA-**3'**
rno-miR-146a-5p (reverse)	**5'**-GGGATTCTGGAAGATGATGATGACTGAGAACTGAA-**3'**
Universal Primer (forward)	**5'**-GGACGGTAGCAAGCAAAGAGTGTG-**3'**
Universal Primer (reverse)	**5'**-GGGATTCTGGAAGATGATGATGAC-**3'**

TABLE 2. The three resulting target genes of rno-miRNA-146a-5p retrieved from miRSel database.

Sr.	Target gene	Gene description
3	Irak1	interleukin-1 receptor-associated kinase 1
4	IL1b	interleukin 1 beta
7	Traf6	Tnf receptor-associated factor 6 (predicted)



Graphical Abstract

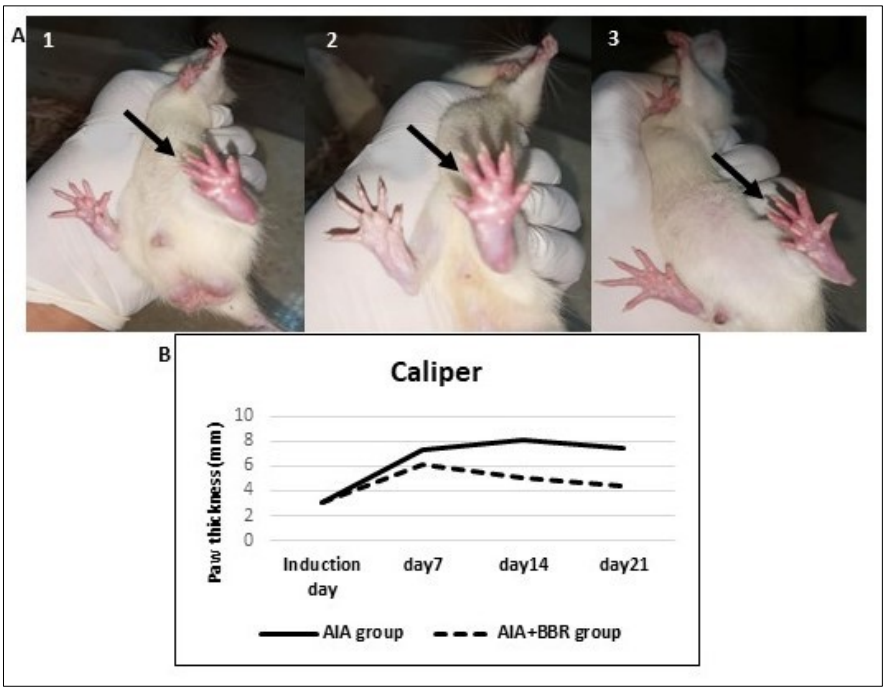


Fig. 1. (A) Paw photographs from arthritic groups showing paw inflammation. Photographs 1: from AIA group at day 7 showed red swollen inflamed left paw (black arrow) compared to non-injected right paw which was more observed at day 14 in photograph 2. Photograph 3: from AIA+BBR treated group showed ameliorated paw. (B) Graph for paw swelling thickness. AIA: Adjuvant induced arthritis group, AIA+BBR: Adjuvant induced arthritis treated with berberine group.

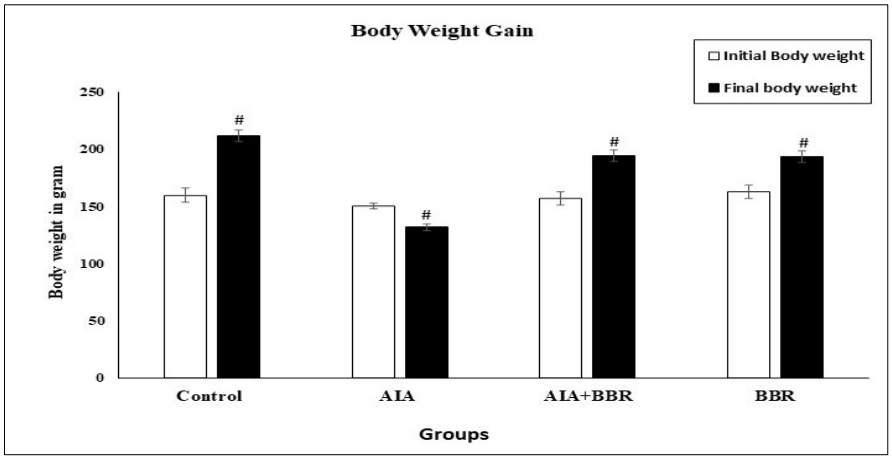


Fig. 2. Bodyweight gain in animals of different experimental groups. # significant change compared to initial body weight, *P*< 0.05. AIA: Adjuvant induced arthritis group, AIA+BBR: Adjuvant induced arthritis treated with berberine group. BBR: Berberine treated group.

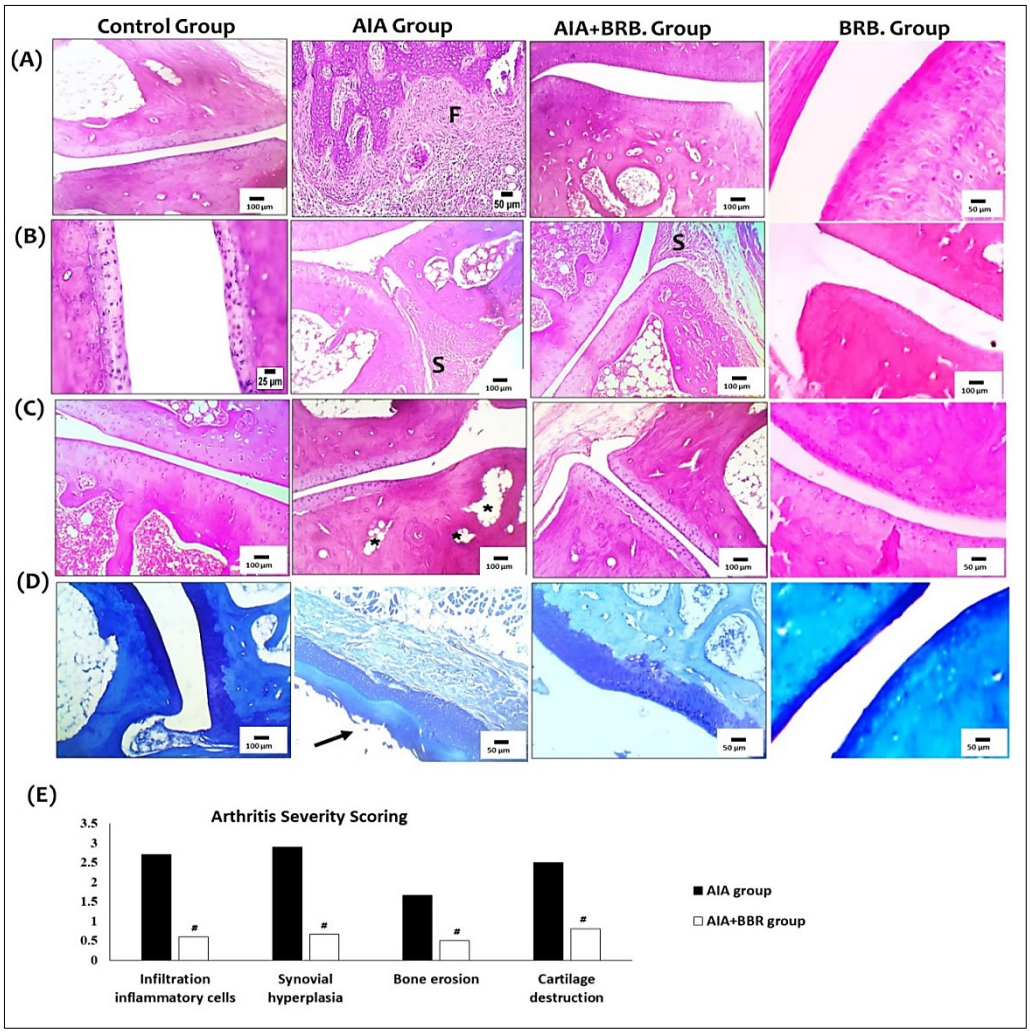


Fig. 3. Arthritis severity scoring criteria of different experimental groups in joint sections stained with H&E (A, B&C) and toluidine blue (D). A: showed inflammatory cells infiltration (F). B: showed synovial hyperplasia (S). C: showed bone erosion (*). D: showed cartilage destruction (arrow). E: showed arthritis severity histological scoring. # significant change compared to AIA group, *P*< 0.05. AIA: Adjuvant induced arthritis group, AIA+BBR: Adjuvant induced arthritis treated with berberine group.

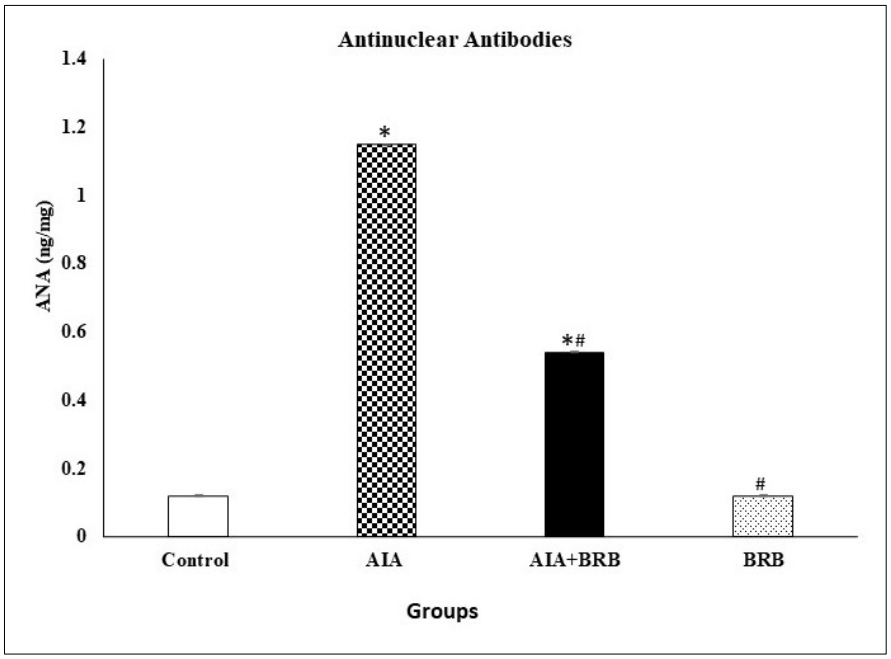


Fig. 4. Levels of antinuclear antibodies in spleen tissue. AIA: Adjuvant induced arthritis group, AIA+BBR: Adjuvant induced arthritis treated with berberine group, BBR: Berberine treated group. Statistically significant at P<0.05; *: compared to control group; #: compared to AIA group; n=6.

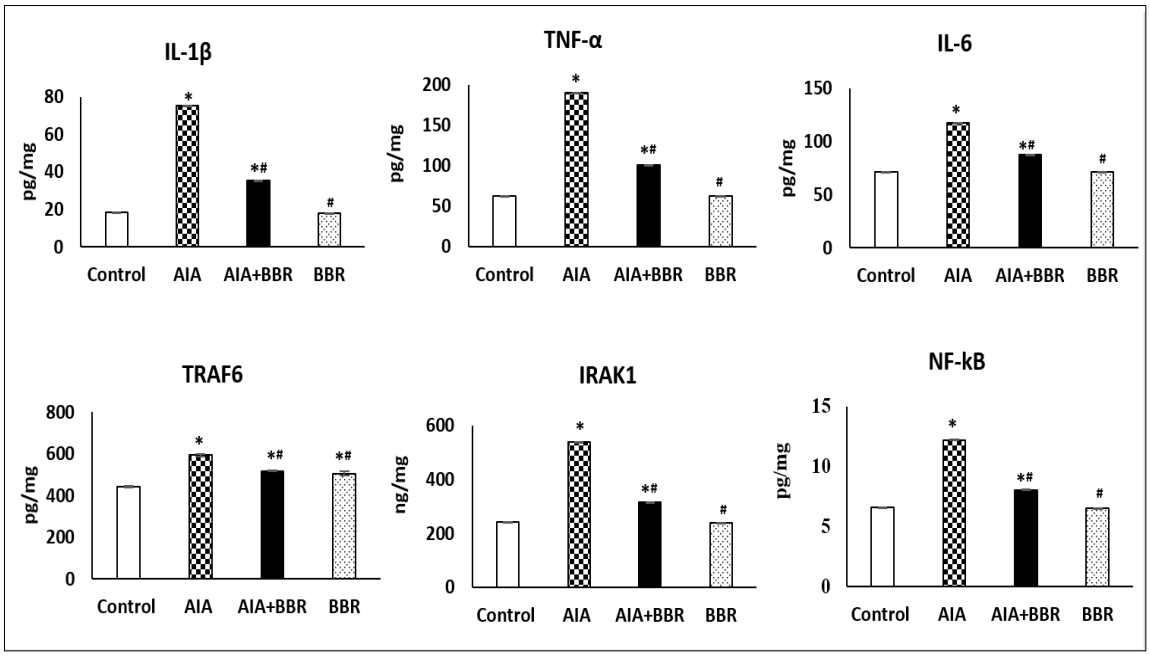


Fig. 5. Levels of IL-1, TNF-α, IL-6, TRAF6, IRAK1 and NF-κB in spleen tissue. AIA: Adjuvant induced arthritis group, AIA+BBR: Adjuvant induced arthritis treated with berberine group, BBR: Berberine treated group. IL-1: interleukin-1, TNF-α: tumour necrosis factor-alpha, IL-6: interleukin-6, TRAF6: TNF receptor-associated factor 6, IRAK1: IL-1 receptor associated kinase 1, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cell. Statistically significant at P<0.05; *: compared to control group; #: compared to AIA group; n=6.

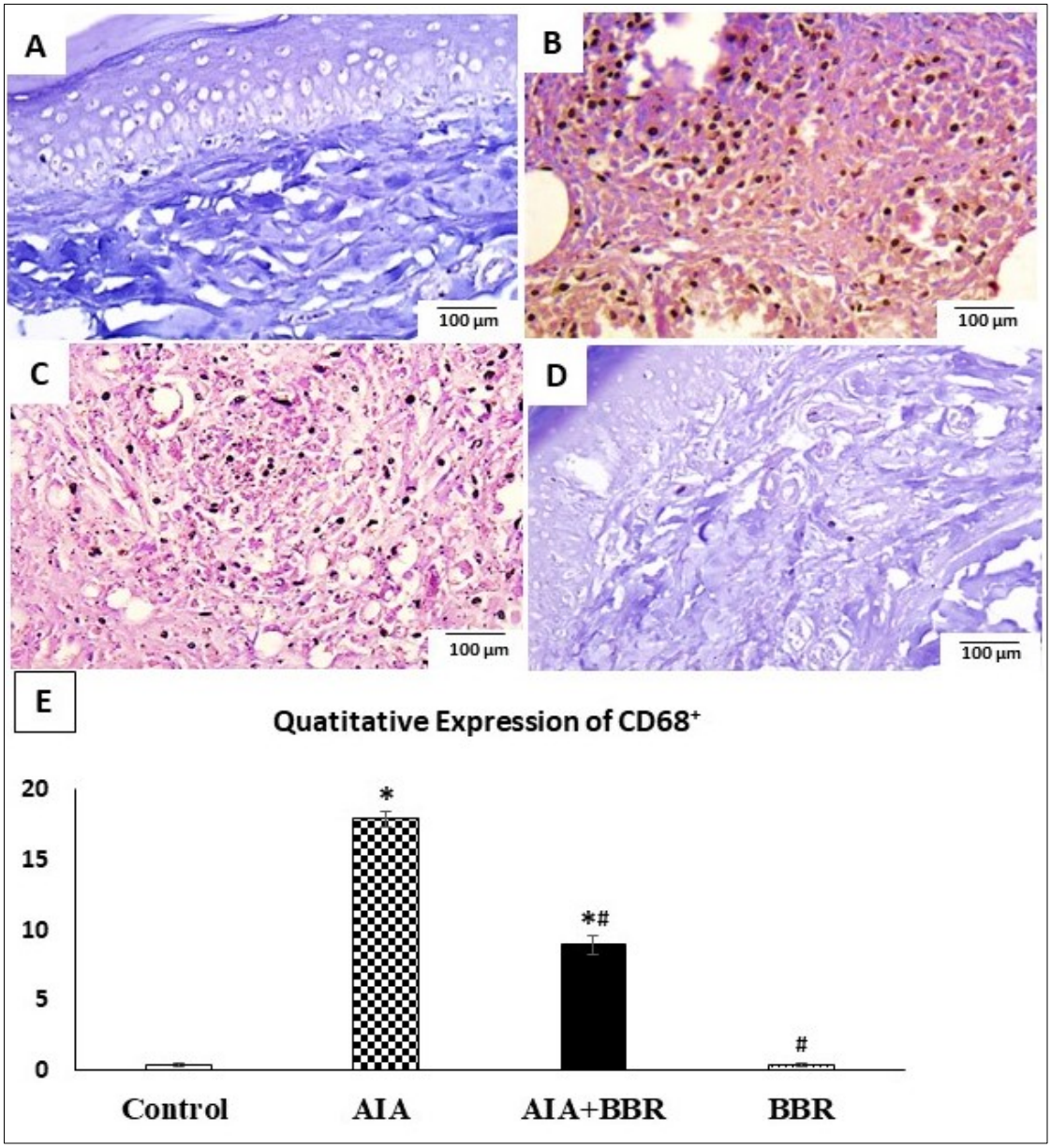


Fig. 6. Effect of BBR on the expression levels of CD68+ immune cells in joint sections. (A&D): showing low expression of CD68+ immune cell reactivity in control and BBR groups respectively. (B): AIA group showing high expression of CD68+ immune reactive cells (brownish in colour). (C): AIA+BBR groups showing moderate expression of CD68+ immune reactive cells. (E): Quantitative analysis of CD68+ macrophages in different experimental groups. AIA: Adjuvant induced arthritis group, AIA+BBR: Adjuvant induced arthritis treated with berberine group, BBR: Berberine treated group. Statistically significant at P<0.05; *: compared to control group; #: compared to AIA group; n=6.

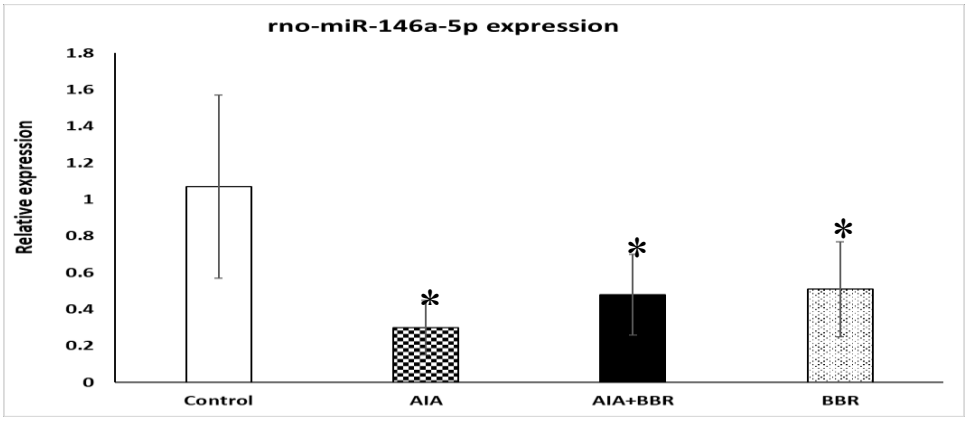


Fig. 7. Effect of BBR on rno-miR-146a-5p expression levels. Triplicates were used in the reaction and analysed *via* qRT-PCR. AIA: Adjuvant induced arthritis group, AIA+BBR: Adjuvant induced arthritis treated with berberine group, BBR: Berberine treated group. Statistically significant at P<0.05; *: compared to control group; #: compared to AIA group; n=6.

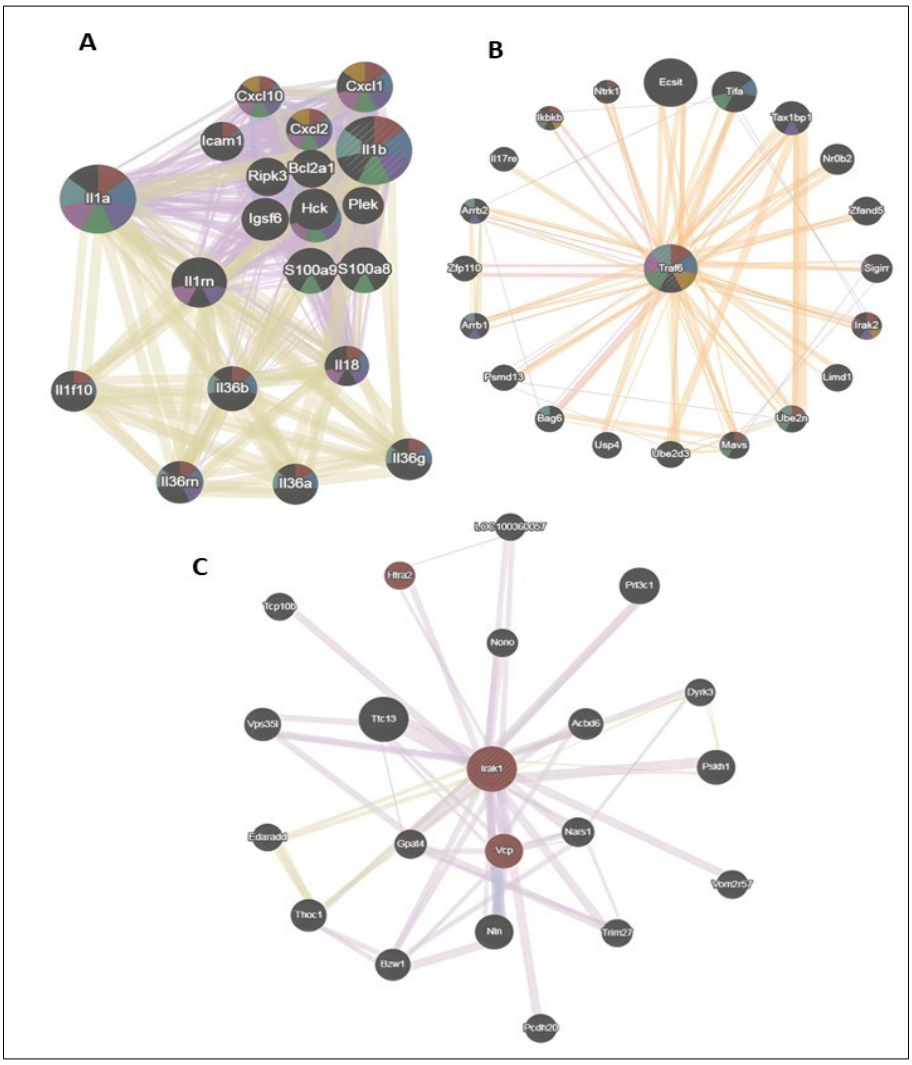


Fig. 8. Genes network maps (A): Il1b gene; (B) Traf6 gene; (C): Irak1 gene; using GeneMANIA bioinformatics tool. The predicted genes are placed in the outer circles while the hub gene is positioned in the inner circle

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التداخل بين miRNA-146a-5p ومسار TRAF6/IRAK1/NF-κB في إلتهاب المفاصل المحدث بالعامل المساعد أدجوفند: التركيز على استخدام البربرين كمنظم للإلتهاب

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الملخص

لقد اكتسبت الميكرو رنا شهرة مؤخرًا بسبب أدوارها الحاسمة في تنظيم العديد من العمليات البيولوجية. ووجد أن تعبيراتها مرتبطة بالعديد من الإضطرابات الإلتهابية مثل إلتهاب المفاصل الروماتويدي. إلتهاب المفاصل الروماتويدي هو اضطراب مناعي ذاتي مزمن يمكن أن ينشأ بسبب عوامل جينية وما فوق الجينات. البربرين هو قلويد مشتق من الإيزوكينولين وله تاريخ طويل من الاستخدام الطبي. يتمتع بفعاليات واسعة مضادة للالتهابات والبكتيريا والأكسدة. ومع ذلك، لم يتم دراسة تأثير البربرين على مستوى تعبير الميكرو رنا-146 (miR-146a-5p) حتى الآن. وبالتالي، فإن الدراسة الحالية هي التقرير الأول الذي يستكشف الآلية الدقيقة للبربرين في تعديل تعبير miR-146a-5p في نموذج إلتهاب المفاصل المحدث بالعامل المساعد أدجوفند .تم تقييم شدة التهاب المفاصل باستخدام تلطيخ الهيماتوكسيلين-إيوزين وتلطيخ التولويدين الأزرق. علاوة على ذلك، تم إجراء تلطيخ مناعي كيميائي لتعبير CD68+ في الخلايا البلعمية. تم قياس مستوى تعبير-miR 146a-5pبواسطة تفاعل البوليميراز المتسلسل الكمي اللحظي (qRT-PCR). أظهرت النتائج المستخلصة انخفاضًا في تعبير miR-146a-5p مرتبطًا بزيادة مستويات الأجسام المضادة للأنوية الذاتية، TNF-α، IL-1β، عامل النسخ النووي كابا ب(NF-κB) ، IL-6، كيناز المستقبل المرتبط بالإنترلوكين-1(IRAK1) ، العامل المرتبط بمستقبل TNF 6 (TRAF6) والتعبير الكمي للبلعمات CD68+ في نموذج إلتهاب المفاصل المحدث بالعامل المساعد أدجوفند فى الجرذان. من الجدير بالذكر أن علاج البربرين يستعيد مستويات جميع هذه الوسائط. بشكل جماعي، كشفت النتائج الحالية عن الدور المحتمل لـ miR-146a-5p في تنظيم مرض إلتهاب المفاصل من خلال التلاعب بإشارة TRAF6/IRAK1/NF-κBكمسار التهابي للخلايا البلعمية. علاوة على ذلك، أظهر البربرين قدرة محتملة على استعادة مستويات تعبير miR-146a-5p وتعديل بروتينات مسار NF-κB في نموذج إلتهاب المفاصل المحدث بالعامل المساعد أدجوفند فى الجرذان.

الكلمات الدالة:CD68+ ، المفصل، الخلايا البلعمية ، إلتهاب المفاصل ، الطحال.